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ON CHANGES IN THE RELATIVE WEIGHTS OF THE VISCERA AND OTHER ORGANS FROM BIRTH TO MATURITY—ALBINO RAT

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In 1912 Jackson and Lowrey (6) showed that the musculature of the growing albino rat increased in its relative weight from 24.4 per cent at birth to 45.4 per cent at maturity. The proportion of the body weight represented by the four other divisions,—the integument, the ligamentous skeleton, the viscera and the remainder—decreased in every case between these limits, though in all, except the remainder, there was a slight rise before the end of the suckling period, this rise coinciding with a slight fall in the value of the musculature.

That the musculature contributes most to the increasing weight of the growing body is shown also by the data for man and other mammals. That coincidently the relative weight of the viscera decreases with increasing body weight is generally recognized (2). However, owing to the physiological importance of the internal organs, a further analysis of the data seemed desirable. The organs designated as "viscera" by me are listed in table 1, while the remaining organs there entered are grouped separately. After each organ is given the body weight or age at which the maximum relative percentage weight occurs.

1. On the relative percentage weights of the viscera and other organs. The data used have been derived from the tables for the several organs in Donaldson (3) and Hatai (5) and the methods of preparing and weighing the organs are given there.

On the body weights for each sex, as given in these tables at each millimeter of body length, the percentage weights of the individual organs have been computed. When the values for the twelve organs here grouped as viscera, table 1, are summed and the percentages computed at a series of selected body weights, the results as given, table 2, are obtained.

TABLE 1

List of organs with the body weights at which the maximum relative percentage weights are found

ORGANS INCLUDED IN VISCERA*	BODY WEIGHT OF "MAXIMUM"		OTHER ORGANS	BODY WEIGHT OF "MAXIMUM"	
	grams			*grams*	
Lungs	4.9	Birth	Blood	4.9	Birth
Hypophysis	5.1	Birth			
Thyroid	5.1	Birth	Eyeballs	7.5	
Submaxillaries	5.8		Brain	11.4	
			Spinal cord	11.4	
Kidneys	7.5				
Heart	9.4		Thymus	53.6	
Pancreas	15.9				
Spleen	18.5		Testes	100.0	
Suprarenals	25.0		Ovaries	122.7	
Liver	25.0		Epididymis	178.0	
Intestinal tract	31.1				
Stomach	34.9				
All viscera (12)	25.0				

* In this study the term "viscera" is used merely as a designation of convenience and not as a precise term. The list of organs used by me as viscera differs from that of Jackson and Lowrey (6) by containing the submaxillaries and hypophysis and by lacking the brain, spinal cord, eyeballs, thymus and gonads. The alimentary tract of Jackson and Lowrey includes the stomach, pancreas and intestinal tract. My list differs from theirs in naming these parts separately.

My list of organs differs also from that used in *The Rat* (3) for table 73, in containing the submaxillaries and in lacking the gonads and the thymus. The alimentary tract as given in table 73 is equivalent to the stomach, pancreas and intestinal tract combined.

The data for the weight of the intestinal tract are still in manuscript form.

TABLE 2

Weight of viscera (12) as in table 1

BODY WEIGHT	MALES		FEMALES	
	Weight of viscera	Percentage on body weight	Percentage on body weight	Weight of viscera
grams	*grams*			*grams*
4.9	0.534	10.89	11.12	0.545
25.0	4.808	19.23	19.24	4.811
50.0	8.872	17.74	17.73	8.865
100.0	14.942	14.94	14.94	14.944
200.0	24.784	12.39	12.41	24.816
300.0	33.574	11.19	11.23	33.678
400.0	42.050	10.51	10.76	43.059

From table 2 it would be possible to chart directly the percentage values for the viscera and thus picture the changes in their relations with increasing body weight. The course of change can however be indicated more conveniently by using the initial percentage as a standard and computing the ratios of the succeeding percentages to it. As this

TABLE 3

Relative percentage weight of viscera

BODY WEIGHT	MALES	FEMALES
	Relative per cent	Relative per cent
grams		
4.9	1.00	1.00
25.0	1.76	1.68
50.0	1.64	1.55
100.0	1.38	1.31
200.0	1.14	1.08
300.0	1.03	0.98
400.0	0.97	0.92

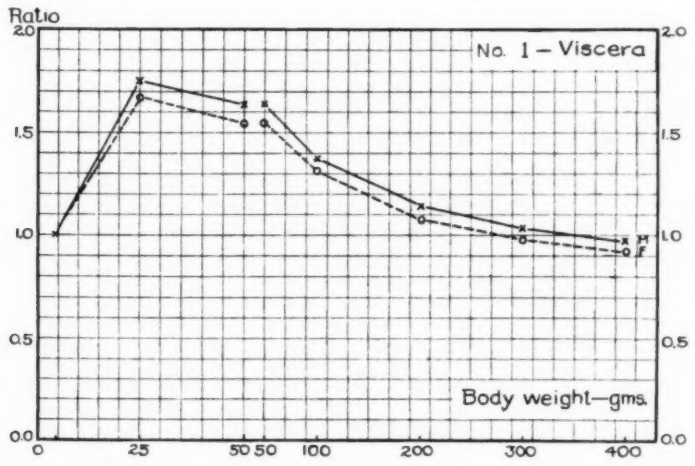


Chart 1. Showing the relative percentage values for the weight of the viscera at a series of selected body weights. x————x, males; o----------o, females.

latter method facilitates the comparision between the several viscera, when these are considered individually, it has been adopted here. By treating the percentage values in table 2 in this way the ratios for the viscera as given in table 3 were obtained. These are also presented in chart 1.

Chart 1 shows that the relative percentage weight for the viscera reaches a maximum at the end of the suckling period (25 grams) and then diminishes steadily. As will be seen from table 2, the percentage values are nearly

TABLE 4

Relative percentage weight of organs on body weight

BODY WEIGHT	LUNGS		HYPOPHYSIS		THYROID		BLOOD		SUBMAXILLARIES	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
grams										
4.7–5.1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
6.2	—	—	—	—	—	—	—	—	—	1.03
25.0	0.62	0.57	0.78	0.71	0.87	0.90	0.84	0.87	0.85	0.85
50.0	0.50	0.47	0.61	0.57	0.76	0.79	0.79	0.81	0.74	0.74
100.0	0.42	0.39	0.50	0.62	0.66	0.68	0.73	0.75	0.62	0.62
200.0	0.37	0.35	0.41	0.49	0.56	0.58	0.67	0.75	0.56	0.56
300.0	0.35	0.33	0.38	0.71	0.51	0.53	0.63	0.72	0.53	0.53
400.0	0.34	0.32	0.36	0.73	0.48	0.50	0.62	0.70	0.50	0.50

BODY WEIGHT	EYEBALLS		KIDNEYS		HEART		BRAIN		SPINAL CORD	
4.7–4.9	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
7.5	1.08	—	1.59							
8.0	—	1.07	—	—	—	1.09				
9.4	—	—	—	—	1.11					
9.9	—	—	—	1.56						
11.4	—	—	—	—	—	—	1.52	—	1.15	
12.0	—	—	—	—	—	—	—	1.58	—	1.13
25.0	0.73	0.70	1.38	1.34	1.03	1.00	1.09	1.11	1.06	1.03
50.0	0.49	0.50	1.17	1.12	0.89	0.88	0.65	0.65	0.85	0.83
100.0	0.34	0.33	1.01	0.97	0.76	0.73	0.36	0.34	0.62	0.60
200.0	0.24	0.23	0.91	0.88	0.64	0.63	0.20	0.20	0.43	0.42
300.0	0.19	0.18	0.87	0.84	0.59	0.58	0.14	0.14	0.34	0.33
400.0	0.15	0.15	0.85	0.82	0.56	0.55	0.11	0.11	0.27	0.26

BODY WEIGHT	PANCREAS		SPLEEN		SUPRARENALS		LIVER		VISCERA	
4.7–5.1	—	—	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
5.8	—	—	—	—	1.10					
6.2	—	—	—	—	—	1.14				
11.4	1.00									
12.0	—	1.00								
15.9	1.18									
16.8	—	1.09	—	1.88						
18.5	—	—	1.80							
25.0	1.08	1.04	1.72	1.82	1.15	1.11	1.81	1.81	1.76	1.68
50.0	0.85	0.90	1.67	1.76	0.89	0.97	1.67	1.66	1.64	1.55
100.0	0.70	0.73	1.56	1.65	0.67	0.85	1.41	1.41	1.38	1.31
200.0	0.60	0.61	1.50	1.59	0.52	0.78	1.17	1.17	1.14	1.08
300.0	0.56	0.55	1.44	1.53	0.45	0.75	1.06	1.06	1.03	0.98
400.0	0.53	0.52	1.44	1.53	0.41	0.74	0.99	0.99	0.97	0.92

TABLE 4—*Concluded*

BODY WEIGHT	INTESTINAL TRACT		STOMACH		THYMUS		GONADS		EPIDIDYMIS
	Males	Females	Males	Females	Males	Females	Testes	Ovaries	Males
grams									
4.7–4.9	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
7.5	—	—	—	—	1.50				
8.0	—	—	—	—	—	—	—	3.00	
9.9	—	—	—	—	—	1.57			
11.4	—	—	—	—	1.36	—	5.75	—	0.78
14.3	—	—	—	—	—	1.36			
15.9	—	—	—	—	—	—	5.38		
25.0	4.27	3.77	1.43	1.41	1.68	1.57	6.08	2.19	0.90
29.1	—	3.81							
31.1	4.34								
34.9	—	—	1.56						
36,9	—	—	—	1.53					
50.0	4.13	3.61	1.50	1.49	2.10	2.10	9.96	2.00	1.33
53.6	—	—	—	—	2.14				
56.8	—	—	—	—	—	2.14			
62.9	—	—	—	—	—	—	—	1.00	
100.0	3.46	3.08	1.23	1.22	1.86	1.92	16.25	3.00	2.55
122.7	—	—	—	—	—	—	—	4.00	
178.0	—	—	—	—	—	—	—	—	3.67
200.0	2.79	2.44	0.94	0.92	0.84	0.57	13.58	1.95	3.63
300.0	2.46	2.16	0.78	0.79	—	—	11.18	2.00	3.22
400.0	2.26	1.98	0.71	0.70	—	—	9.58	1.00	2.71

the same for the two sexes but since the initial percentage is slightly greater for the female, the subsequent ratios for the female run somewhat below those for the male, but at the moment it would be unwise to attach any particular significance to this difference, although it is in harmony with the precocity of the female at this stage.

The relations to which attention is called are the maximum relative percentage weight of the viscera at 25 grams—the end of the suckling period—and the steady decrease in the relative percentage as the rat increases in body weight.

These relations are here given for the viscera as a group, and we now pass to the consideration of the individual organs comprised as viscera and also of the other organs listed in table 1.

By the use of full tables (not given here) for the absolute and the percentage weights of each organ under consideration, and by taking the percentage weight at birth as unity, the relative percentage weight for each organ was determined in each sex for a series of selected body weights in the same manner as for the viscera—just presented. The results are grouped in table 4.

Table 4 presents the data for the twenty organs taken individually and also for the viscera as a group. The first entry is for the organ (lungs) showing the greatest steady decrease in the relative percentage weight. This is followed by the cases giving progressively higher final values. Then come those which show a post-natal rise in the relative percentage weight beginning with the submaxillaries in which the rise occurs earliest i.e., at the lowest body weight) and progressing to the case in which it occurs latest (epididymis). For this arrangement the data for the male are taken as a guide, but the female follows approximately the same course except for the hypophysis and the suprarenals.

In constructing the tables the following adjustments were made. The initial body weights range from 4.7 to 5.1 grams, but these are not separately indicated for the individual organs. All the initial values are for these body weights, save in the case of the pancreas for which, owing to the uncertainties of dissection, the initial values are for body weights

TABLE 5

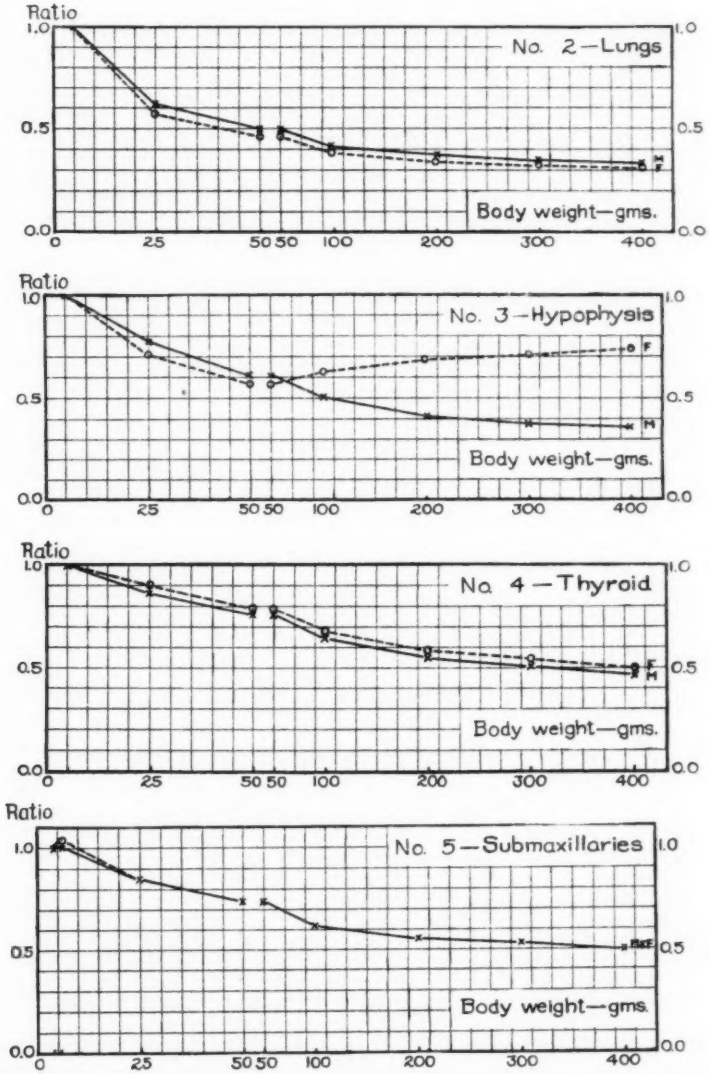
Relations of body weight to age in the series of data used for this study

PHASE	BODY WEIGHT		AGE
	Male	Female	
	grams	*grams*	*days*
Birth	4.9–5.1	4.7–5.0	0
Beginning activity	7.6	7.5	5
Returns to mother	13.0	12.0	10
Eyes open	17.0	18.0	16
Weaning	21–23	22.5–24.5	22–24
Puberty	105–150	98–135	70–90

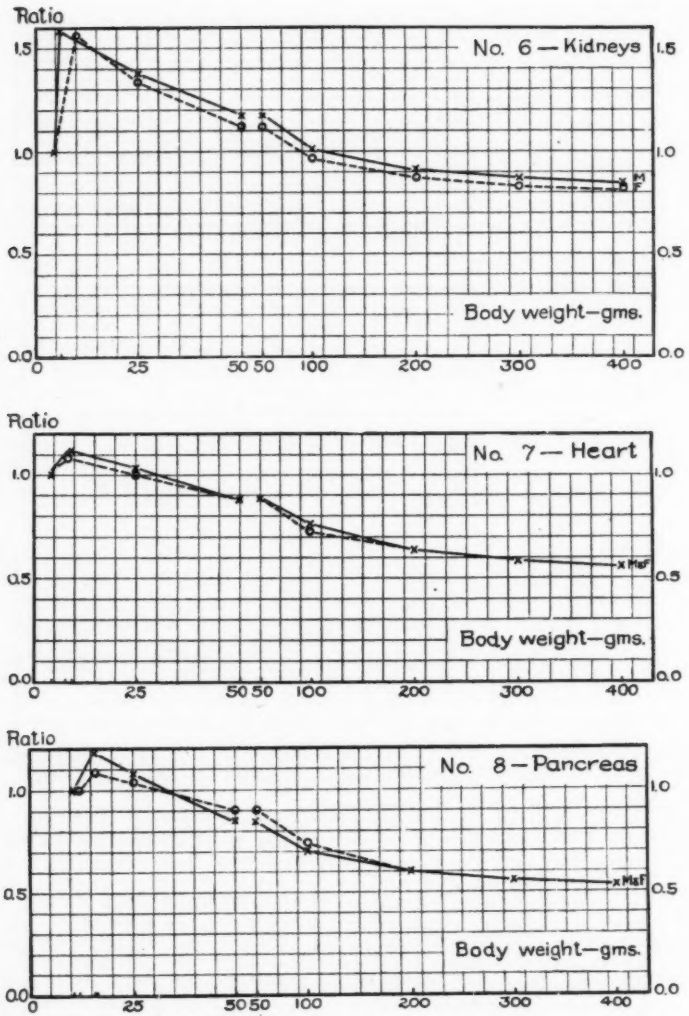
at 11.4 grams, male, and 12.0 grams, female. In some cases the course of the relative percentages fluctuates and the high and low values which thus appear are entered at the body weights to which they apply.

Applicable to *this series* of data are the following relations between body weight and age. (Table 5.)

The accompanying charts give in graphic form the same relations as are shown in table 4 and in the same order, save that chart 1 for the viscera—which has already been given—is here omitted. The charts are in general similar in construction save that the scale of values on the ordinates is reduced in the cases where the increase in relative values is large. The scale on the abscissa is changed in every chart at 50 grams and the value for 50 grams is repeated one division farther on. For the first 50 grams, the value of a division is 5 grams, after this it is 20 grams. This change in scale must be borne in mind when interpreting the later portion of each chart. The male values are indicated by x————x. The female values by o————o. Where the two sexes give values which run close together only the male graph is drawn.



Charts 2 to 21. Showing the changes in the relative percentage weights of the twenty organs—based on the data in table 4. x————x, males; o----------o, females.



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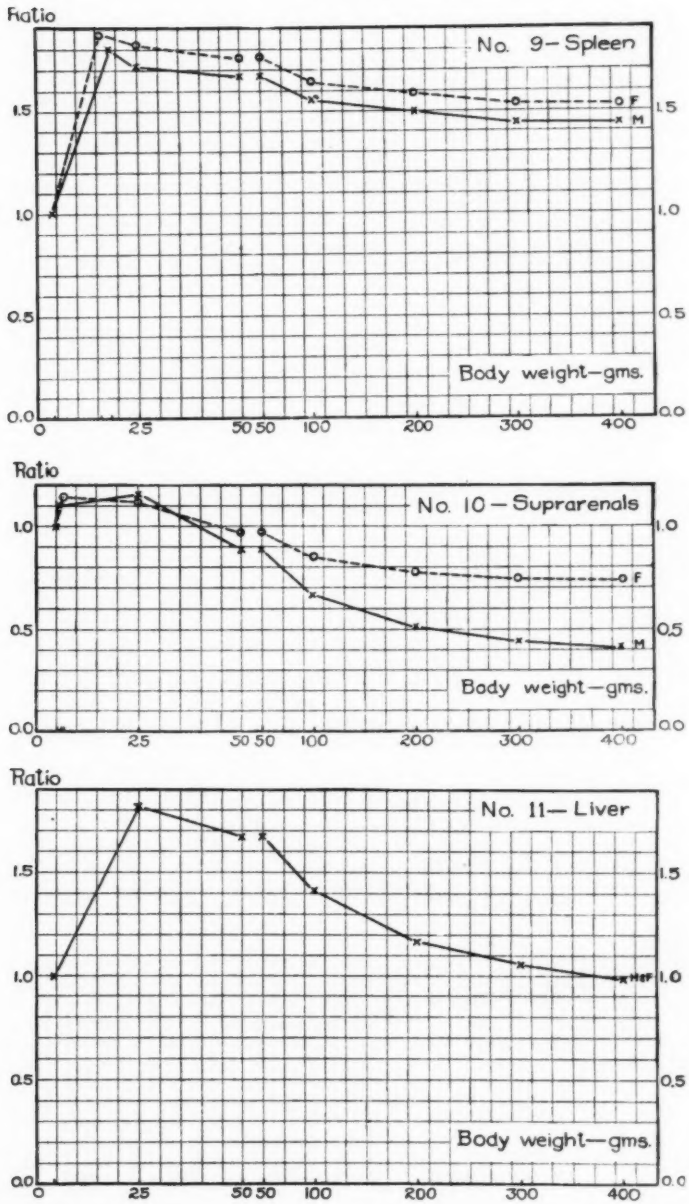

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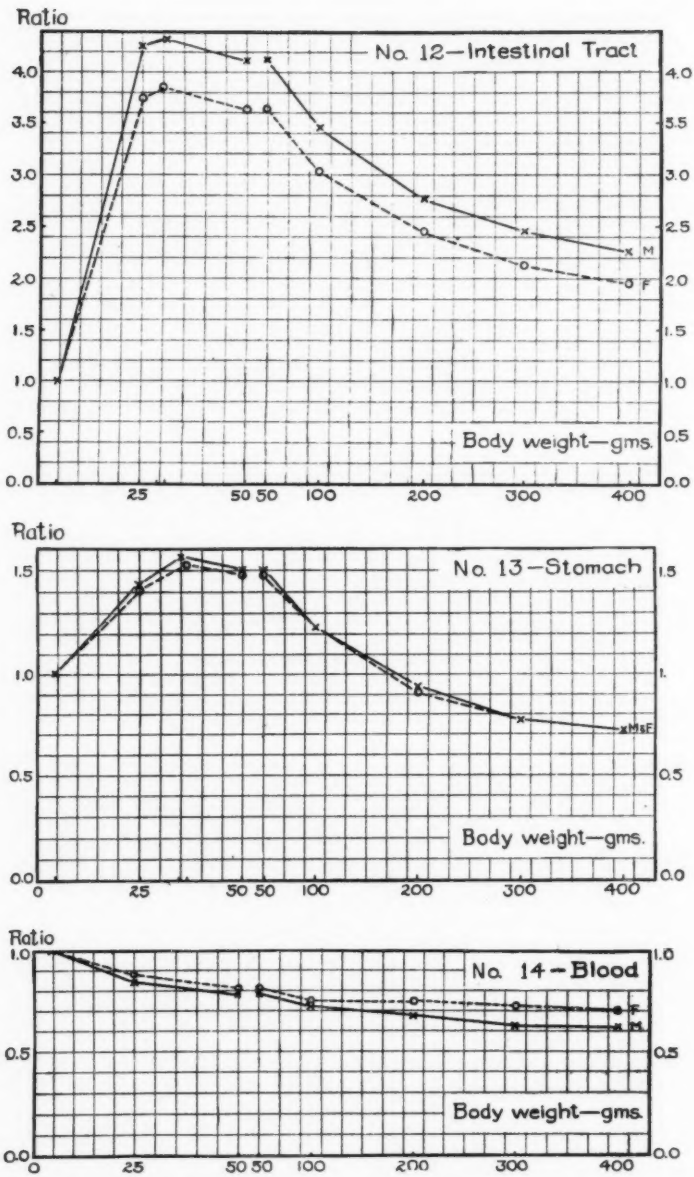

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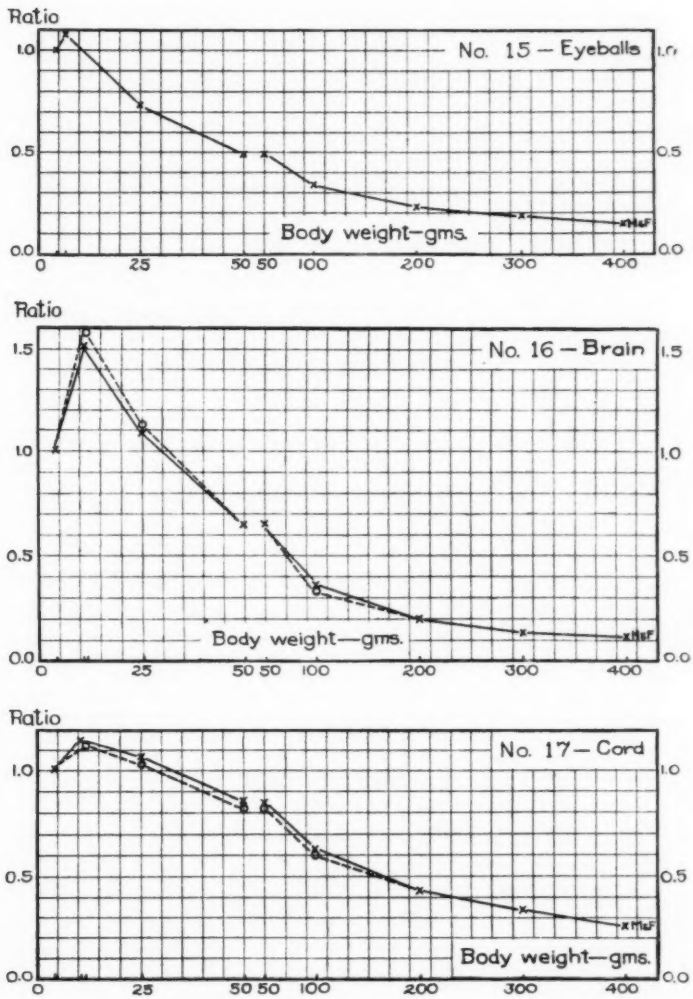

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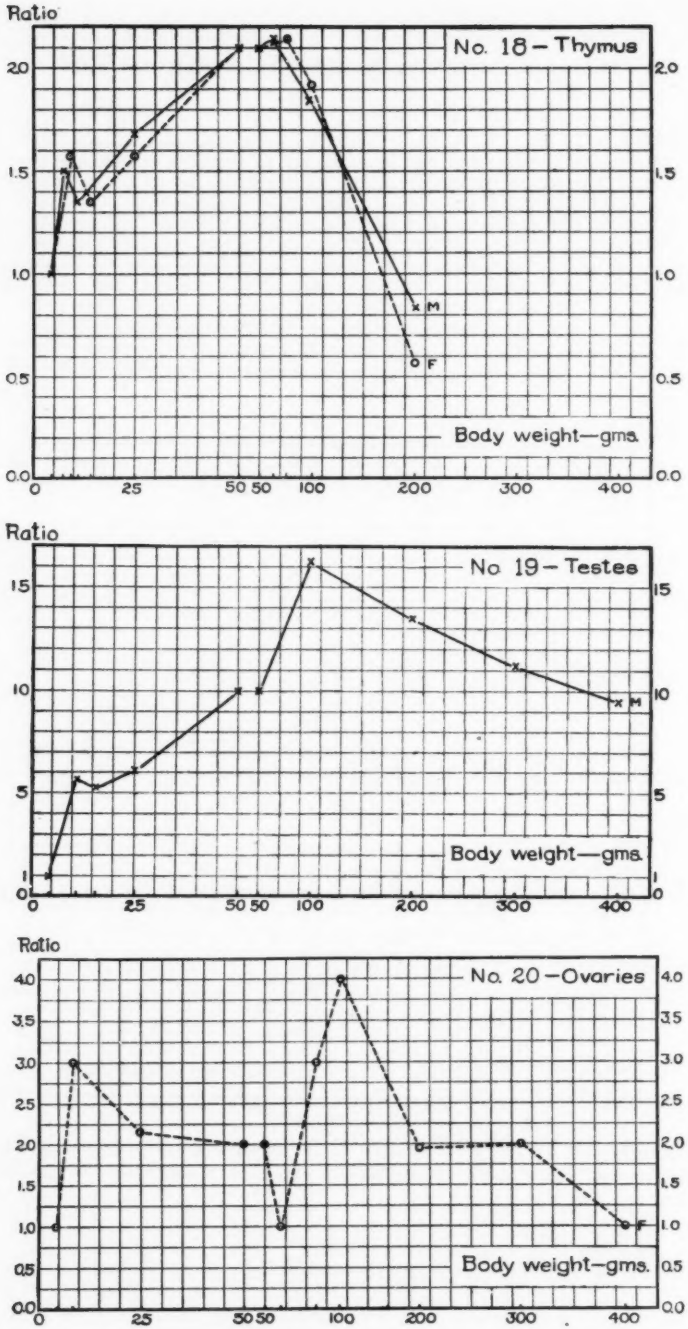

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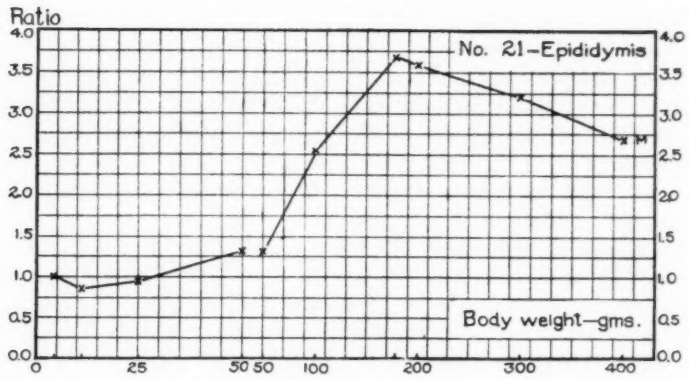

Charts 2 to 21. Showing the changes in the relative percentage weights of the twenty organs—based on the data in table 4. x————x, males; o---------o, females.

As to the differences according to sex which appear in the charts, those shown by the hypophysis and suprarenals are clear and marked—but in the case of the other organs in which a difference appears, any interpretation should wait on more detailed study.

The main facts revealed by these charts are as follows: The presence of a well-marked maximum in the relative percentage weights of the organs —a maximum which marks the stage at which the organ is growing most rapidly in relation to the growth of the entire rat and the appearance of this maximum at different body weights from 4.7 grams (birth) to 100 grams (puberty) or later.

Taking the organs in series and using the increasing body weight as the measure of progress, the data show that the lungs and blood—the oxidizing organs—have their respective maxima at birth as do also two glands related to growth—the hypophysis and the thyroid.

Between birth and 11.4 grams come in order the maxima for the submaxillaries, eyeballs, kidneys, heart and the central nervous system. The maxima for the heart and kidneys are related to beginning locomotion.

The brain and spinal cord at 11.4 grams represent the directing system and reach their respective maxima about the time the young rat begins to wander (10 days).

Then follow, between 15.9 and 34.9 grams, the alimentary organs and others, in the order of pancreas, spleen, suprarenals, liver, intestinal tract and stomach, all in close relation to weaning and its consequences. Next appears the maximum for the thymus—standing apart—at 53.6 grams, but coincident with the inception of the period of rapid body

growth; and finally the gonads at 100 grams or more show maxima associated with puberty.

Although the foregoing account has been given in terms of body weights, yet its significance is in terms of age as connotated by the body weights.

When interpreting these relations it must be kept in mind that, with the exception of the thymus, all of the organs here considered increase in absolute weights throughout life (see graphs (3)). Even the thymus grows for some time after attaining its maximum relative percentage weight.

This maximum occurs therefore when the organ is small and does not relate to its absolute size, but merely to its rate of growth in relation to that of the entire rat. The observations show that just previous to coming into function each organ exhibits a period of relatively rapid growth. Such a period must be present in the case of every organ and in those instances, just cited, in which no post-natal maximum appears this event occurs during fetal life. There is then during development a wandering wave of accelerated growth passing from organ to organ and reaching its full expression at different times in different organs but tending to appear in a given organ shortly before its activity becomes well marked. Or to transpose this statement—well-marked activity does not occur until the period of accelerated growth is attained.

Jackson and Lowrey (6) called attention to the well-known wave of growth which progessses from the head caudad, and if one examines table 1 for the body weights at which the maxima occur, the same relations appear in a very general way.

2. On the absolute weight increase of the organs. It is desirable in this connection to examine in more detail the absolute growth of the viscera in relation to the growth of the entire rat.

If the increase over the birth weight of the body and the corresponding increase in the weight of the viscera are tabulated as in table 6 (based on the weights given in table 2), several interesting relations appear. Thus while the body weight increases (from 4.9 to 400 grams) 81.6 times, the the weight of the viscera between these limits increases very nearly as many times.

In the absence of further analysis it might be said that the rat at 400 grams had the viscera-body relations found at birth,—a case of second childhood.

Table 6 shows that between birth and 400 grams the viscera are always increasing faster than the body, save in the last instance, and that this increase is most marked at the 25 gram stage.

This means of course that while both body and viscera increase nearly the same number of times between birth and 400 grams, yet the rate of growth in the viscera is so distributed as to give them the highest per-

TABLE 6

Comparing the ratios of the body weights and of the corresponding weights of viscera as given in table 2, using the initial weight as unity in each instance

BODY WEIGHT	MALE AND FEMALE RATIOS OF BODY WEIGHT	RATIO OF VISCERA WEIGHTS	
		Males	Females
grams			
4.9	1.0	1.0	1.0
25.0	5.1	9.0	8.8
50.0	10.2	16.6	16.2
100.0	20.4	28.0	27.4
200.0	40.8	46.4	45.5
300.0	61.2	62.8	61.8
400.0	81.6	78.7	79.0

TABLE 7

Using like body weights for the two sexes at birth—4.9 grams—the number of times the birth weight was gained at 400 grams is recorded for both sexes, within groups, in the order of the appearance of the relative percentage weight maxima. Owing to lack of data the thymus is not entered here

ORGAN	MALES			FEMALES		
	Weight at		Increase (times)	Weight at		Increase (times)
	Birth	400 grams		Birth	400 grams	
	grams	*grams*		*grams*	*grams*	
Lungs	0.0780	2.1580	28	0.0800	2.1630	27
Hypophysis	0.0005	0.0130	26	0.0005	0.0294	59
Thyroid	0.0015	0.0556	37	0.0015	0.0556	37
Submaxillaries	0.0170	0.7050	41	0.0170	0.7050	41
Kidneys	0.0460	3.1870	69	0.0480	3.1800	66
Heart	0.0310	1.3930	45	0.0320	1.3910	43
Pancreas*	0.0400	1.6900	42	0.0350	1.7960	51
Spleen	0.0090	1.0330	114	0.0090	1.0350	114
Suprarenals	0.0017	0.0547	32	0.0017	1.0020	59
Liver	0.2100	17.090	81	0.210	17.090	81
Intestinal tract	0.0700	12.926	184	0.080	12.820	160
Stomach	0.0300	1.745	58	0.030	1.792	59
Viscera (H.H.D.)	0.5340	42.050	78.7	0.545	43.059	79.0
Eyeballs	0.0290	0.386	13	0.029	0.385	13
Brain	0.2260	2.044	9	0.217	2.015	9
Cord	0.0330	0.734	22	0.034	0.748	22
Testes	0.0040	3.053	763			
Epididymis	0.0047	0.994	212			
Ovaries				0.0008	0.524	65
Blood	0.4400	22.300	51	0.4300	24.35	57

*The initial body weight for the pancreas is, males 11.4 grams and females 12 grams.

centage at 25 grams, as shown in table 2, and after that a decreasing percentage value at each of the periods is noted—with the consequence that at 400 grams the percentage weight of the viscera is like that at birth. A little futher detail will clarify this set of relations.

In table 7 the increase for each organ between birth and 400 grams is given. The first group of twelve organs, from lungs to stomach inclusive, is that here designated as *viscera*. If one notes the increase of these organs as given in the column headed "increase: times" and keeps in mind that, as shown by the entry for the viscera, these increase 78.7 times (in the male) then it appears that among the viscera only three organs—the spleen, liver and intestinal tract show an increase above that of the viscera as a whole—while the other organs, like the lungs and ductless glands, for instance, show much less. Nevertheless the absolute bulk of the liver and intestinal tract is so great and the increase in the intestinal tract is so large that they overbalance the other organs and determine the relations found. The analysis may be followed one step further. If the rate of increase in the weight of the intestinal tract is computed at the selected body weights the values appear as in table 8.

TABLE 8

Increase in the weight of the intestinal tract—male and female

MALES			FEMALES	
Body weight	Weight of intestinal tract	Increase (times)	Weight of intestinal tract	Increase (times)
grams				
4.9	0.070	1.0	0.080	1.0
25.0	1.528	21.8	1.528	19.0
50.0	2.958	42.3	2.914	36.4
100.0	4.968	71.0	4.972	62.2
200.0	7.986	114.1	7.890	98.6
300.0	10.553	150.8	10.431	130.4
400.0	12.926	184.7	12.823	160.3

This shows a large and steady increase in the weight of the intestinal tract in both sexes after 100 grams of body weight. The result of this long continued increase is to give a percentage weight for the intestinal tract at 400 grams which is about twice the initial percentage weight—(see intestinal tract, table 4.

Among the other organs grouped as viscera only the spleen shows a final percentage weight above the initial value, while the liver, which forms a large fraction of the viscera, drops a point below its initial value.

These relations may be briefly summarized as follows: Between birth and 400 grams the viscera increases in weight nearly as many times as does the entire rat. The percentage weight of the viscera at 400 grams is

close to that at birth. The growth of the viscera is precocious, however, so that they have a high percentage value at 25 grams and from that body weight on the percentage diminishes. These relations of the viscera to the body are dominated by two out of the twelve organs here considered—namely, the liver and the intestinal tract, which by reason of their large absolute weights determine the results. The intestinal tract is the more important. At 400 grams it has about twice the percentage weight which it had at birth—while the liver has only about the birth percentage, and the other viscera, with the exception of the spleen, show only from 0.82 to 0.15 of the initial percentage. By reason of its small absolute weight the spleen has been omitted from the discussion.

It follows from this that although at 400 grams the viscera represent the same percentage of the weight of the entire animal that they did at birth, yet the proportional values of the twelve organs comprised in the viscera are very different in the two instances. Some of the consequences of this alteration in the weight interrelations of the organs may now be considered.

3. On the weight interrelation of the several organs. The data before us make possible an examination of the relative weights of the organs, *a*, in relation to the anatomical divisions of the body, and *b*, in relation to the blood.

a. Jackson and Lowrey (6) have divided the entire rat into the integument, musculature, skeleton, viscera and a remainder. As stated at the beginning of this paper, the later increase in body weight is most largely due to the growth of the musculature which doubles its percentage value between birth and maturity; while, as a consequence of this, the values for the integument, skeleton, remainder and viscera all diminish from an early maximum, and thus the relative value for the viscera is reduced by the greater growth of the other portions of the rat—especially the musculature. This result naturally raises the question concerning the physiological status of the rat when the relative weight of the viscera is thus reduced. A consideration of the physiological demands of those portions which the viscera are required to regulate and maintain is thus called for.

In approaching the problem we take departure from the fact that primarily growth is accomplished by cell multiplication—followed by cell enlargement—but accompanied also by the formation of fat, keratin, salts, myelin, fluids and connective tissue which though adding to the weight of the rat are relatively inactive substances. The proportion of these tends to increase with the increasing size of the rat.

Futher, the rate of cell multiplication diminishes from birth on, and later growth, where it is not due to mere deposit, as for example salts in the bones or myelin in the nervous system, is the result of cell enlargement.

Cell multiplication becomes insignificant or ceases at an early age both in the musculature (7) and the nervous system (1) continues for the time in the long bones, persists in the integument in relation to the growth of hair and the epidermis, and is of course more or less continuous in the organs comprised in the viscera, the blood forming tissues, lymph glands and gonads.

As the maximum relative weight of the viscera occurs at about 25 grams of body weight and as later growth of the rat is mainly growth by cell enlargement, it seems proper to associate the high development of the viscera with this form of growth, keeping in mind of course the need of all the completed cells for maintenance. The demand for visceral activity lessens as the rate of growth diminishes.

In this connection attention is called to the fact that the hypophysis—a regulator of cell mutiplication—decreases in relative weight from birth on, as does growth by this process.[1]

Also the thymus is at its maximum relative weight just before the period of most rapid body growth which in this series occurs from 50 to 70 days. This rapid growth is most probably due to cell enlargement and there thus appears to be a time relation between the percentage maximum for this gland and cell enlargement.

b. Weight relations of the organs to the blood. Between these relatively reduced viscera on the one side and on the other the greater mass of the body which in the mature rat calls for maintenance—the blood acts as an intermediary.

As the data show, the relative weight of the blood diminishes with the increasing weight of the animal so that at 400 grams a unit weight of tissue receives only 0.62 as much blood as goes to the same unit weight at birth. From the standpoint of the intensity of the physiological processes the conditions at maturity are plainly less advantageous for the animal as a whole than they are at birth. To measure the amount of this disadvantage is not at present possible, but it may be safely said that owing to the reduced physiological activity of the great bulk of the mature rat, the disadvantage is less than the gross weight relations would suggest.

If now the blood is considered in its dependence on the viscera, some relations of interest appear. From a maximum of 1.00 at birth the blood weight drops to 0.62 at 400 grams. As the blood is the organ which is mainly responsible for the internal environment of the tissues, it is of interest to determine how its composition might be modified by changes in those organs which contribute to it.

[1] The less rapid decrease in relative percentage weight in the case of the female albino rat depends of course on the greater growth of the hypophysis in the female of this form, but is peculiar to it—as it does not occur in the wild Norway (4).

Looked at from this angle it appears that the hypophysis (final relative value 0.36 male; 0.73 female) the thyroid (final relative value 0.48 male; 0.50 female) and the suprarenals (final relative value 0.41 male; and 0.74 female) diminish in the males faster than does the weight of blood—whereas in the female the diminution in the case of the hypophysis (see footnote to table 1) and of the suprarenals is less than that of the blood.

From these weight relations it is inferred that in the larger male rats the blood is less well supplied with the secretions from these glands—while in the female the condition has changed less. The relations of the suprarenal in the female must be passed over for the present.

Moreover the weight of the heart and of the lungs diminishes more rapidly than does that of the blood, and the mechanical distribution and oxidation of the blood appears therefore to be less well cared for. This analysis suggests that while in the relations of the organs to the blood the differences appearing with increasing body weight are much less than those which develop between the viscera and the remainder of the body, nevertheless even the blood suffers in its composition and distribution in the larger rat, which is therefore in this respect also at a disadvantage by reason of its size.

If now we turn to the alimentary group—submaxillaries, pancreas, liver, intestinal tract, stomach and kidneys—as furnishing metabolites to the blood and removing wastes from it, the computations show that these organs, taken together, have at 400 grams only 50 per cent of their maximum value and by inference the composition of the blood is less favorable for physiological processes in the large rat than in the small one.

SUMMARY

This survey of the organ relations is here limited to be the period between birth and maturity—the last phase of development—but it is evident that between the formation of the embryo and birth a corresponding study would yield very different results, depending on the age limits which were chosen.

In view of the fact that in pharmacological studies and nutrition experiments the organ composition of the rat enters as a factor, and that this composition alters with the body growth (age) of the rat, it seemed worth while to make a survey of the organ relations in this animal between birth and maturity.

This has been done here for a series of twenty organs, with special reference to those designated as viscera, on the assumption that the weight of the organ is an index of its physiological value to the rat. It is recognized, of course, that this assumption can be valid only in a broad way. In the interest of a comprehensive view, however, the relations considered have been merely sketched.

Thus the weight of the blood is dealt with as though the histology and chemistry of the blood were the same from birth to maturity, which they are not. Nevertheless the general picture of the organ relations made possible by this method of presentation has a value, despite its limitations.

Under the heading "viscera" is given in table 1 a group of twelve organs which may be classed together and under the "other organs" several other groups—including the blood.

In table 4 and charts 2 to 21 the percentage weight of the organ at birth is taken as unity, and then for a selected series of body weights the relative percentage weight is determined. This shows when the greatest relative percentage weight occurs and indicates when the organ in question is growing most rapidly in relation to the growth of the body.

The greatest relative percentage weight is designated as the maximum, and it appears that this maximum is found for the several organs forming the viscera from birth to a body weight of 34.6 grams while in the group designated "other organs," the range is from birth to 170 grams. The maximum as thus determined tends to appear just before, or at the time, the functional activity of the organ becomes important.

Nevertheless between birth and 400 grams the weight of the viscera increases nearly as many fold as does the weight of the entire rat, so that the percentage value of the viscera is about the same at 400 grams as at birth. Since, however, the phase of rapid growth for the viscera culminates at 25 grams, the percentage value rises to this stage—but afterwards falls steadily. A study of the increase in the weight of the organs comprised in the viscera reveals that it is mainly the intestinal tract and the liver, which by their large size determine the relations found, and further analysis shows that although the percentage values for the viscera are the same at the two limiting body weights, yet the weight interrelations of the organs concerned are very different.

Since in relation to the body as a whole the viscera after 25 grams become relatively lighter with increasing body weight and at 400 grams have only about 50 per cent of their maximum relative value, it appears that measured in this way the larger animal is at a physiological disadvantage, but this disadvantage is in a measure compensated by the fact that the portions of the body to be regulated and maintained probably make smaller physiological demands in the larger than they do in the smaller rat.

When the relation of the organs to the blood is considered it is seen that, in the males at least the ductless glands become less adequate with increasing body weight, as do also the remaining viscera, though in the latter instance, the disparity is decidedly more marked. Further, the organs concerned with the circulation and oxidation of the blood,—the heart and the lungs—decrease relatively faster than does the weight of the blood itself, so that in this respect the larger animal is again at a disadvantage.

The general outcome of these comparisons indicates that in any series of studies on the rat, the age and weight should be known in order to make possible an estimation of the organ composition of the animal—a matter of importance—since this organ composition is a modifying factor affecting the results obtained.

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Note: Through an oversight, proper recognition has not been made of the work of C. M. Jackson (Journal of Anatomy, xv, 1913) which shows in percentages the relations of the organ weights on body weight. For this regrettable omission, I beg to apologize to the author.

THE RELATION OF MINUTE GLUCOSE OUTPUT TO MINUTE VOLUME OF PERFUSION IN THE ISOLATED LIVER OF THE TERRAPIN

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Snyder and Martin (1) perfusing the isolated, surviving liver of the terrapin, studied vascular reactions of the organ to changes in C_H and epinephrin. Snyder, Martin and Levin (2) observed the effect of these factors on the rate of glucose output. In both investigations the minute volume of perfusion was accurately determined, as was the minute weight output of glucose. Discussing the necessity for considering the time element in perfusion experiments of this nature, the authors state: "The results of the present investigation show that the determination of mere percentage of reducing substances in untimed samples of liver outflow furnishes no reliable data concerning the absolute amount of that substance delivered up to the general circulation. In problems of hepatic glycogenesis or glycogenolysis minute output determinations appear to be indispensable."

By employing this method these authors (2) discovered that there is a general relationship between rate of perfusion and rate of glucose output, ". . . . usually wherever the volume outflow is increased there also the sugar output per unit of time increases and vice versa." This may be explained on the basis of mass action. The perfusion period was probably too short in most cases to allow observations on this point to be at all decisive, for ". . . . while the change in the sugar output tends to follow in the same direction as that of the volume flow per unit of time, the amount or extent of the change in the two factors do not seem to bear any relation to each other."

I have been particularly interested in showing that there is a distinct and regular relationship between volume of perfusion and glucose output. It seemed to me that with a perfect experiment one might show a constant output of sugar for any given volume flow, provided of course the store of glycogen and of diastase remained sufficient.

At first, in spite of the precautions to isolate the liver from uncontrollable influences, as internal secretions of adrenals and pancreas, nervous

impulses, etc., and to maintain the hydrostatic pressure of perfusion at a constant level,[1] we found unaccountable variations in the perfusion rate and the glucose output. An example will illustrate:

From experiment of December 15, 1922, Ringer's pH 7.5. Pressure 8 cm. of water

SAMPLE NUMBER	MINUTE VOLUME OUTFLOW PER MINUTE	MINUTE WEIGHT OUTPUT OF GLUCOSE PER MINUTE	CONCENTRATION GLUCOSE
	cc.	*mgm.*	*per cent*
8	11.62	1.38	0.0117
9	11.60	0.696	0.006
10	11.55	0.738	0.0064
11	14.22	2.14	0.0152
12	13.82	2.63	0.0119

In the experiment of February 24, 1923, using Ringer's of pH 6.6, perfusion rates were varied by raising the Mariotte bottle to a higher level after collection of each three or four samples. The greatest care was taken to prevent leakage in the system. To this end as little dissection was done as possible. Ample time, 10 to 30 minutes, was allowed after each change in pressure, that equilibrium might be attained between volume of flow and sugar formation. With a stop-watch the time for collection of successive 5 cc. samples was recorded. Only when the rate of perfusion had reached a fairly constant level was a total sample of 15 or 25 cc. retained for analysis. Several samples were taken at each pressure. These were analyzed separately. Analysis was performed with greatest care, using a routine of procedure (3) the error for which, as shown previously, by analysis of a weighed sample of glucose, was less than 3 per cent for the range of concentrations usually encountered. Figures 1 and 2 present the results of the experiment in graphic form. The order of collection of samples is indicated by the numerals 1 to 25 placed opposite the coördinates. The values distribute themselves along a fairly regular curve. Even though relatively large fluctuations occur at any perfusion level, as for samples *4*, *5*, *6* and *7*, it is significant that all these values fall along the curve. That they would not do so if time were not allowed for equilibrium to be established before taking the samples is attested by the results of numerous former experiments whose curves were extremely irregular.

Values *20* to *23* inclusive fall, unaccountably, far below the general level of the curve. One might suppose that 16 cm. of pressure, under these particular conditions had, in some way, retarded glycogenolysis, possibly by mere compression of the cells beyond their normal limit.

It is extremely interesting to note how number *24*, after return to 8 cm. pressure and interval for equilibrium establishment of 30 minutes, falls

[1] See the article by Snyder and Martin (1) for details of the method of perfusion.

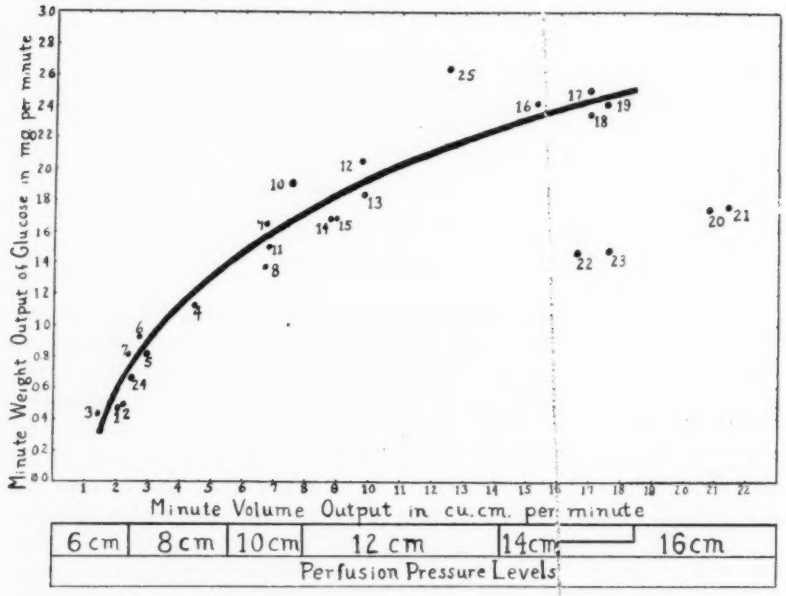

Fig. 1. Curve showing that minute volume output, controlled by changes in perfusion pressure, affects the minute weight output of glucose in a regular manner. Ringer's pH 6.6. Duration of perfusion, 5 hours.

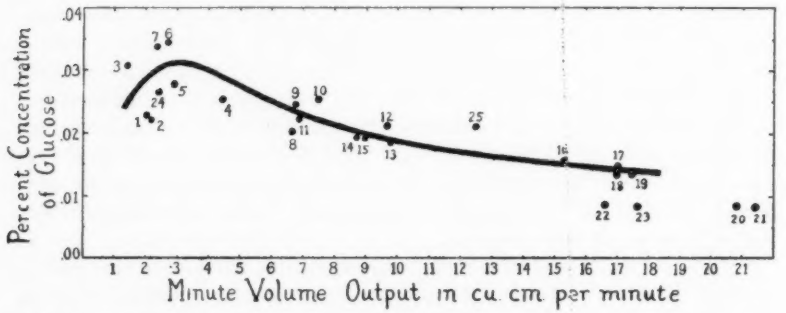

Fig. 2. Curve showing the lack of a constant relation between per cent concentration of glucose and the minute volume flow. Same experiment as in figure 1.

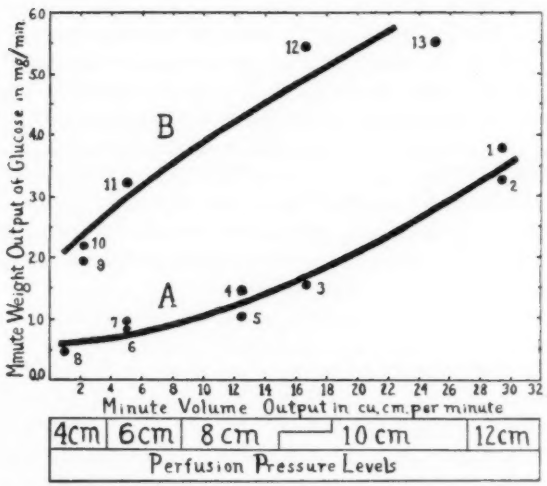

Fig. 3. *A*. Normal relation between volume flow and glucose output. *B*. Increased rate of glucose output with epinephrin 1:10^8 added. Ringer's pH 6.6. Duration of perfusion, 2½ hours.

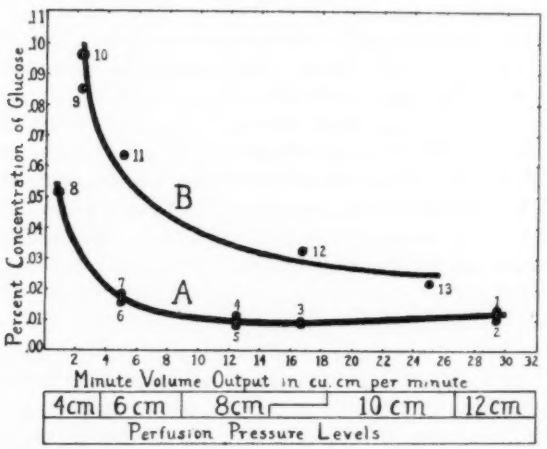

Fig. 4. Curves of glucose concentration for the epinephrin experiment, showing that lowest concentration may occur with highest minute output of sugar, and vice versa.

almost exactly in line with *4*, *5*, *6* and *7*, collected at the same pressure more than 2 hours previously. Number *25* was collected at 12 cm., allowing only 6 minutes for equilibration at this new pressure.

Figure 2 shows the concentrations of glucose in the perfusates. It is interesting to note that as the perfusion rate is increased there is at first an increase in concentration of the glucose. Further increase in the rate, however, is attended by a very gradual decrease in concentration, though, as figure 1 has shown, the minute output is then continually increasing. This emphasizes that, although there is a constant relationship between minute volume of perfusion and minute output of sugar, there is not necessarily a constant relation between minute volume of flow and the concentration of glucose in the perfusate.

Increasing the perfusion pressure is not the only factor that will increase the volume flow and the rate of glucose output. Epinephrin, as shown by Snyder, Martin and Levin (2), when in minimal effective concentration ($1:10^9$) and with pH lower than 7.3, causes an increased perfusion rate and an increased glucose output. They expressed doubt as to whether epinephrin increases sugar output merely as a consequence of greater irrigation of the liver, or whether the drug accelerates glycogenolysis per se. Figure 3 shows the results of an experiment to determine this point. Ringer's solution of pH 6.6 was used. Curve *A* represents the normal relation between minute volume outflow and output of sugar, as in figure 1. Curve *B* represents the same relationship after addition of epinephrin ($1:10^8$) to the perfusing fluid. It is clear that at any given volume flow the sugar value is much higher with epinephrin than without. Presumably, then, epinephrin accelerates glycogenolysis.

In figure 4 are represented the various glucose concentrations encountered in the epinephrin experiment. One sees that the concentration falls rapidly as the perfusion rate increases. The concentration at 4 cm. (sample *8*) for Ringer's alone is more than twice as great as for Ringer's plus epinephrin at 10 cm. pressure (sample *13*). Arguing from concentrations alone one would assume that epinephrin retards glycogenolysis under these conditions. But considering the rate of glucose output in figure 3, we see that the above conclusion is erroneous and that the rate of glycogenolysis for epinephrin at 10 cm. is eleven times that for Ringer's alone at 4 cm. This experiment again emphasizes the necessity of considering minute output as well as percent concentrations.

It should be stated here that in speaking of glucose output from the perfused liver we have no proof that this glucose is being produced by glycogen hydrolysis. It might conceivably have been present from the start as free glucose. Glycogen analyses were not made nor were determinations of the free glucose performed, for it was found that the total amount of glucose washed away during a whole experiment could not

exceed 1 or 2 grams. Loss of the corresponding amount of glycogen could not be accurately detected by present methods of analysis. Yet, since epinephrin is known to produce hyperglycemia, and hence certainly to promote glycogenolysis in the intact animal, and since this drug appears to increase the minute output of sugar from the perfused liver, it would be natural to suppose that here also we deal with a process of glycogenolysis.

It is our hope to continue the studies with epinephrin. As yet we have no conclusive experiment, by the present method, with perfusions of high pH (above 7.3) for minimal epinephrin. With a high pH the perfusion is very slow (1) and minimal epinephrin retards the rate even further. As much as 90 minutes may be required to reach the equilibrium point with low pressures.

SUMMARY AND CONCLUSION

1. Former work (2) has shown that in perfusion experiments for the study of glycogenolysis:

a. The minute volume of perfusion and the minute weight output of glucose should be determined rather than merely the per cent concentration of this substance.

b. A general, though not always constant, relation appeared to exist between minute weight output of glucose and minute volume outflow. Increase in the latter was usually accompanied by increase in the former, and vice versa.

2. Evidence is now offered (fig. 1) from one of a series of eight experiments that, where perfusion minute volume is regulated by changes in pressure level, there is a constant and regular relationship between minute sugar output and minute volume of perfusion.

3. Data are given (figs. 2 and 4) showing the lack of a simple relation between percent concentration of glucose and minute volume. Comparison with figures 1 and 3 shows strikingly the impossibility of measuring the rate of glucose output in terms of concentration alone.

4. Five experiments, one of which (using epinephrin $1:10^{8}1$) is cited, show that in studying the effect of any factor on glycogenolysis, where there is any reason to suspect that that factor will also influence the perfusion rate, the constant relation between perfusion minute volume and output of sugar must be taken into account. This may best be done:

a. By ascertaining the approximate relationship of these factors for the liver used, by preliminary perfusion covering a fairly wide range of minute volumes.

b. By then perfusing at one or more pressure levels, using the drug to be studied. If this drug affects glycogenolysis per se, the curve of the new relationship between minute volume outflow and minute sugar output will fall above or below the normal curve, according as to whether the substance accelerates or retards glycogenolysis.

5. It is suggested that, by employing the methods outlined above, the effect on glycogenolysis of other drugs, of inorganic salts, and even of nerve impulses might be advantageously studied.

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STUDIES OF THE THYROID APPARATUS. XVIII

The Differential Development of the Albino Rat from 100 to 150 Days of Age and the Influence of Thyro-Parathyroidectomy and Parathyroidectomy Thereon

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Development is the composite of that progressive series of changes in size and structure which takes place in the living organism from the moment of its conception until its termination as a physiological unit. It is more than growth because it is the processes of development which give rise to the structural type of organism characteristic of the species, while growth is simply the increase in size or number, or both, of the structural units.

Just as it is permissible to consider the differences between species as sequelae of the differences in the specific developmental processes, so it is permissible to consider the anatomical and functional differences of the various parts of an organism as the sequelae of differences in the developmental processes within the individual. In last analysis specific differential development is but the expression of the differential development of the individual.

Differential development is both qualitative and quantitative. The former determines structural type; the latter, structural size. A study of quantitative differential development within an individual is thus a study of the relative growth of the various parts. It is an evaluation of the relative intensity of the development of the different organs of the body and a foundation for the determination of the factors concerned in proportionate and disproportionate development. It is the latter phase of development that is of chief interest in the present study.

Proportionate differential development gives rise to a group of individuals the members of which, although they may differ from each other in absolute size, do not differ in the relative size or shape of their body parts. It is a matter of common observation that in any group of individuals obtained by random sampling there is rarely evidence of uniformity of bodily proportions. The individuals of such a group, when compared with each other, give evidence that disproportionate differential

development has occurred within the species. Since the fitness of the individual for the environment is somewhat related to his structural make-up, an investigation of the causes of disproportionate development should be of value.

The previous papers of this series have been concerned with the detailed report of the growth of the individual structures as such and the rôle of the thyroid apparatus therein (1). Here the individual organs are considered as parts of the whole and in relation to each other in an attempt to picture the influence of thyroid and parathyroid functions on differential development within the organism. There the high-dry lens was used for detail, here the low-power binocular is used for perspective.

The period of growth on which these studies are made was that lying between the ages of 100 and 150 days in the life of the albino rat. According to Donaldson (2) this represents an interval of approximately four years in the life of man between the ages of eight and twelve years. Donaldson's conception is based on his comparative studies of the growth of the central nervous system, and while valid for that system, should be used with caution in an interpretation of the comparative differential development in the two species because of the relative age difference in sexual maturity and the influence of this process on development.

The data which serve as the basis of the present study were obtained from eight groups of rats; four groups of males and four of females. The controls were rats, the gross growth of which was recorded from 100 to 150 days of age at which time the animals were killed and the organ measurements made. The thypars were rats from which the thyroid and parathyroid glands had been removed at 100 days of age and the gross growth studied until 150 days when dissection occurred as with the controls. The parathys were rats deprived of their parathyroid glands when 100 days of age and then treated as the above. The controls were the litter mates of the thypars and parathys. The reference controls were rats which were dissected and the organ measurements taken at 100 days of age. Owing to conditions described elsewhere, the reference controls were not litter mates of the experimental groups, but were from the same stock and had the same original progenitors. The sexes were separate throughout. Diet and environment were the same for all. The details of the plan of the study and the methods of procedure have been given in another place. There are thus had for study records of a series of measurements of various organs at the beginning and at the end of a stated period of growth of normal and test animals the values of which are strictly comparable.

There are two points of view from which the problem can be approached. One, by a study of the weight relationships of the parts to the whole;

two, by a study of the relative growth capacities. Changes in the former with age or age plus experimental procedure are second derivatives since they depend on the relative growth of the individual structure and the total body weight or length. The complexity introduced justifies their non-use in a dynamic interpretation of differential development. On the other hand, certain important correlations are brought out by this method which would otherwise escape attention. I will first point these out, then discuss briefly some general variability relationships that could not have been given in the preceding papers, and use as the chief basis for my discussion the relative growth capacities.

In tables 1 and 3 are given the percentage values of the organ weights in terms of the total body weights of the four groups of male and female rats respectively. Those for the reference controls are arranged in the order of decreasing magnitude. The millimeters of body length per gram of body weight, and the grams of body weight per millimeter of tail, humerus and femur length are also recorded. In tables 2 and 4 are given the grams of organ weight per 100 millimeters of body length, and the percentage values of tail, humerus and femur length in terms of the body length of the same groups.

The relative position in the series of any given organ common to the two sexes is practically the same in the control groups of the males and females. This indicates a specific qualitative similarity in organic make-up.

All the organs save the liver are smaller proportions of the total body weight in the males than in the females even though the absolute weights are greater in the former than in the latter. This holds for the test groups as well as for the controls. That is to say, the processes of development in the female albino rat produce an animal which at the given age is absolutely smaller, but in which the organs of assimilation, transport, direction and excretion are a greater proportion of the total body mass than in the male. The body mass represented by the combined weight of the muscles, skin, skeleton and alimentary tract is both absolutely and relatively greater in the male. It is possible that this sex difference is due to the actually or potentially greater demand made by the female on the organs of vegetative function due to the sex specific processes concerned in reproduction.

It is noteworthy that the relative length of the long bones with respect to the body length is practically the same for both sexes and is not significantly altered by the experimental procedures. This is indicative of a specific, practically sex-indifferent common denominator in the growth in length of sexually homologous skeletal structures. This is not shown in the weight relations.

TABLE 1

The percentage values of the various organs calculated on the total body weight. Males

Age	100				150				150				150			
Group	Reference control				Control				Thypar				Parathy			
Organ	Mean	S.D.	Pem.	C.V.	Mean	S.D.	Pem.	C.V.	Mean	S.D.	Pem.	C.V.	Mean	S.D.	Pem.	C.V.
Liver	4.39	0.55	0.11	12.59	3.63	0.10	0.02	2.78	3.38	0.41	0.08	12.13	3.88	0.36	0.06	9.26
Testes	1.19	0.15	0.03	12.71	0.98	0.09	0.02	9.38	1.15	0.21	0.04	18.55	1.12	0.16	0.03	14.42
Brain	1.08	0.14	0.03	13.08	0.81	0.08	0.02	9.89	1.00	0.19	0.04	19.23	0.97	0.13	0.02	13.60
Kidney	0.77	0.14	0.03	18.63	0.69	0.05	0.01	7.85	0.53	0.05	0.01	9.31	0.62	0.09	0.02	13.99
Lungs	0.61	0.07	0.01	10.59	0.49	0.09	0.02	17.96	0.50	0.13	0.03	26.33	0.51	0.09	0.02	16.57
Heart	0.40	0.03	0.01	8.04	0.35	0.02	0.00	6.36	0.32	0.04	0.01	13.29	0.37	0.02	0.00	5.15
Pancreas	0.39	0.05	0.01	11.96	0.35	0.04	0.01	11.56	0.31	0.07	0.01	22.40	0.37	0.04	0.01	11.44
Epididymis	0.29	0.06	0.01	19.30	0.33	0.03	0.01	8.51	0.35	0.07	0.01	21.09	0.35	0.08	0.01	22.69
Spleen	0.27	0.08	0.02	28.36	0.23	0.04	0.01	15.02	0.20	0.04	0.01	21.40	0.28	0.07	0.01	25.42
Cord	0.27	0.02	0.00	7.92	0.23	0.02	0.00	9.69	0.27	0.05	0.01	18.58	0.26	0.03	0.01	10.65
Fem. wt	0.25	0.02	0.00	6.80	0.23	0.01	0.00	5.19	0.25	0.03	0.01	11.21	0.25	0.02	0.00	9.11
Submax	0.18	0.02	0.01	13.04	0.17	0.01	0.00	5.42	0.16	0.02	0.00	13.63	0.20	0.03	0.01	16.16
Eyes	0.16	0.02	0.00	11.95	0.13	0.01	0.00	10.31	0.16	0.03	0.01	20.94	0.15	0.02	0.00	16.44
Thymus	0.15	0.03	0.01	21.23	0.12	0.03	0.01	21.77	0.09	0.03	0.01	31.32	0.11	0.03	0.00	22.97
Hum. wt	0.11	0.01	0.00	5.26	0.11	0.01	0.00	4.76	0.11	0.01	0.00	10.81	0.11	0.01	0.00	7.27
Thyroid	0.015	0.005	0.001	35.10	0.009	0.002	0.000	24.18					0.009	0.003	0.001	28.57
Adrenal	0.014	0.002	0.000	11.35	0.011	0.002	0.000	14.15	0.011	0.002	0.000	17.27	0.011	0.001	0.000	16.81
Hypophysis	0.0037	0.0004	0.0001	10.81	0.0031	0.0003	0.0001	9.68	0.0047	0.0009	0.0002	19.15	0.0033	0.0002	0.0000	6.06
BL/BW	1.14	0.11	0.02	9.65	0.89	0.09	0.02	10.01	1.07	0.21	0.04	19.59	1.00	0.13	0.02	12.61
BW/TL	1.04	0.09	0.02	8.78	1.33	0.12	0.03	9.08	1.11	0.17	0.03	14.95	1.17	0.16	0.03	13.67
BW/HL	7.06	0.68	0.14	9.63	8.97	0.75	0.15	8.36	7.51	1.26	0.25	16.78	7.96	1.01	0.18	12.63
BW/FL	5.44	0.50	0.10	9.19	6.88	0.62	0.13	9.01	5.80	0.91	0.18	15.68	6.10	0.74	0.13	12.13

S.D., Standard deviation. BL, Body length. HL, Humerus length.
PEM., Probable error of mean. BW, Body weight. FL, Femur length.
C.V., Coefficient of variability. TL, Tail length.
When PEM reads 0.00 or 0.000 the calculated value was found to be less than 0.005 or 0.0005.

TABLE 2

The gram organ weight per 100 mm. of body length and the per cent tail, humerus and femur length values calculated on total body length. Males

Age	100				150				150				150			
Group	Reference control				Control				Thypar				Parathy			
Organ	Mean	S.D.	Pem.	C.V.	Mean	S.D.	Pem.	C.V.	Mean	S.D.	Pem.	C.V.	Mean	S.D.	Pem.	C.V.
Liver	3.86	0.46	0.09	11.89	4.13	0.57	0.12	13.74	3.22	0.58	0.11	17.91	3.88	0.56	0.10	14.32
Testes	1.05	0.12	0.03	11.77	1.11	0.16	0.03	14.20	1.08	0.20	0.04	18.33	1.12	0.17	0.03	14.98
Brain	0.95	0.03	0.01	3.17	0.91	0.03	0.01	3.19	0.93	0.05	0.01	5.47	0.96	0.04	0.01	4.15
Kidney	0.65	0.08	0.02	12.50	0.78	0.11	0.02	14.32	0.51	0.08	0.02	15.75	0.63	0.17	0.03	26.58
Lungs	0.54	0.06	0.01	10.35	0.56	0.11	0.02	19.22	0.47	0.30	0.06	63.61	0.51	0.05	0.01	9.35
Heart	0.35	0.03	0.01	9.12	0.39	0.03	0.01	6.67	0.30	0.03	0.01	9.93	0.37	0.04	0.01	10.59
Pancreas	0.34	0.04	0.01	10.17	0.38	0.05	0.01	13.79	0.29	0.05	0.01	17.36	0.37	0.05	0.01	13.75
Epididymis	0.25	0.05	0.01	21.03	0.37	0.05	0.01	13.67	0.33	0.08	0.02	23.58	0.35	0.09	0.02	25.56
Spleen	0.23	0.05	0.01	20.51	0.27	0.05	0.01	20.00	0.19	0.04	0.01	19.68	0.29	0.09	0.02	32.12
Cord	0.23	0.01	0.00	3.86	0.26	0.01	0.00	4.31	0.25	0.01	0.00	5.72	0.26	0.01	0.00	4.63
Fem. wt	0.22	0.03	0.01	12.67	0.26	0.03	0.01	9.58	0.24	0.03	0.01	11.48	0.25	0.03	0.01	13.78
Submax	0.16	0.03	0.01	17.18	0.19	0.02	0.00	9.63	0.15	0.02	0.00	10.60	0.20	0.04	0.01	21.30
Eyes	0.14	0.01	0.00	4.38	0.14	0.00	0.00	2.82	0.15	0.01	0.00	6.04	0.15	0.01	0.00	5.86
Thymus	0.13	0.03	0.01	23.85	0.14	0.03	0.01	23.57	0.08	0.03	0.01	33.89	0.11	0.03	0.01	25.09
Hum. wt	0.10	0.01	0.00	11.88	0.12	0.01	0.00	8.47	0.11	0.01	0.00	9.52	0.11	0.01	0.00	10.81
Thyroid	0.013	0.004	0.001	30.30	0.010	0.001	0.000	9.80					0.009	0.004	0.001	37.63
Adrenal	0.012	0.001	0.000	10.48	0.012	0.002	0.000	14.17	0.010	0.001	0.000	13.59	0.011	0.001	0.000	12.39
Hypophysis	0.0032	0.0004	0.0001	12.50	0.0035	0.0003	0.0001	8.57	0.0044	0.0007	0.0001	15.90	0.0033	0.0004	0.0001	12.12
BW/BL	88.6	9.1	1.8	10.27	113.3	10.9	2.2	6.96	95.9	15.4	3.0	16.02	101.0	13.0	2.4	12.91
TL/BL	85.4	2.1	0.4	2.46	85.0	2.8	0.6	3.29	85.4	4.1	0.8	4.80	86.7	4.2	0.8	4.84
HL/BL	12.55	0.19	0.04	1.51	12.62	0.30	0.06	2.38	12.67	0.38	0.07	3.00	12.67	0.20	0.04	1.58
FL/BL	16.29	0.49	0.10	3.01	16.47	0.27	0.05	1.64	16.54	0.56	0.11	3.39	16.55	0.36	0.06	2.18

TABLE 3

The percentage values of the various organs calculated on the total body weight. Females

Age	100				150				150				150			
Group	Reference control				Control				Thypar				Parathy			
Organ	Mean	S.D.	Pem.	C.V.	Mean	S.D.	Pem.	C.V.	Mean	S.D.	Pem.	C.V.	Mean	S.D.	Pem.	C.V.
Liver	4.17	0.45	0.10	10.79	3.52	0.34	0.07	9.74	4.25	0.70	0.13	16.51	3.87	0.42	0.07	10.77
Brain	1.23	0.11	0.02	8.56	1.01	0.14	0.03	13.42	1.30	0.22	0.04	16.50	1.22	0.16	0.03	12.99
Kidneys	0.76	0.03	0.01	3.42	0.65	0.09	0.02	13.15	0.62	0.15	0.03	23.60	0.67	0.05	0.01	6.82
Lungs	0.69	0.07	0.02	10.42	0.53	0.12	0.03	23.38	0.62	0.10	0.02	16.67	0.58	0.09	0.02	15.30
Pancreas	0.48	0.06	0.01	13.47	0.40	0.06	0.01	15.19	0.39	0.08	0.01	19.64	0.45	0.08	0.01	18.49
Heart	0.43	0.03	0.01	7.42	0.37	0.04	0.01	10.22	0.39	0.06	0.01	15.50	0.42	0.03	0.01	7.31
Spleen	0.32	0.08	0.02	26.18	0.26	0.04	0.01	13.69	0.23	0.06	0.01	25.11	0.32	0.05	0.01	16.10
Cord	0.30	0.02	0.00	4.97	0.27	0.03	0.01	10.99	0.35	0.06	0.01	16.76	0.32	0.03	0.01	9.03
Fem. wt	0.28	0.02	0.01	7.94	0.26	0.01	0.00	4.25	0.30	0.04	0.01	12.54	0.28	0.02	0.00	7.91
Uterus	0.27	0.09	0.02	33.21	0.27	0.09	0.02	34.43	0.17	0.07	0.01	41.38	0.22	0.13	0.02	58.99
Submax	0.21	0.03	0.01	13.55	0.19	0.03	0.01	13.44	0.21	0.03	0.01	12.56	0.26	0.08	0.01	28.63
Eyes	0.19	0.01	0.00	7.03	0.16	0.02	0.01	14.47	0.21	0.04	0.01	20.39	0.19	0.03	0.01	16.58
Thymus	0.18	0.06	0.01	33.33	0.15	0.03	0.01	19.31	0.10	0.04	0.01	40.63	0.12	0.04	0.01	28.69
Hum. wt	0.13	0.01	0.00	5.56	0.12	0.01	0.00	5.08	0.13	0.02	0.00	14.62	0.13	0.01	0.00	8.73
Ovaries	0.062	0.011	0.002	17.02	0.051	0.010	0.002	19.92	0.046	0.012	0.002	27.02	0.050	0.015	0.003	29.74
Adrenal	0.027	0.006	0.001	21.56	0.023	0.003	0.001	12.45	0.019	0.005	0.001	23.94	0.022	0.004	0.001	18.91
Thyroid	0.015	0.004	0.001	29.33	0.010	0.001	0.000	10.20					0.009	0.001	0.000	10.34
Hypophysis	0.0052	0.0006	0.0001	11.54	0.0059	0.0008	0.0002	13.56	0.0070	0.0006	0.0001	8.57	0.0058	0.0008	0.0001	13.79
BL/BW	1.29	0.08	0.02	6.41	1.09	0.13	0.03	11.82	1.39	0.24	0.05	17.51	1.26	0.15	0.03	11.62
BW/TL	0.90	0.07	0.01	7.21	1.08	0.13	0.03	11.77	0.83	0.14	0.03	17.05	0.91	0.11	0.02	12.28
BW/HL	6.12	0.36	0.08	5.88	7.29	0.89	0.18	12.21	5.81	0.92	0.17	15.83	6.28	0.92	0.16	14.65
BW/FL	4.73	0.26	0.06	5.50	5.68	0.52	0.11	9.15	4.50	0.70	0.13	15.56	4.87	0.56	0.10	11.50

TABLE 4

The grams organ weight per 100 mm. of body length and the per cent tail, humerus and femur length values calculated on total body length. Females

Age	100				150				150				150			
Group	Reference control				Control				Thypar				Parathy			
Organ	Mean	S.D.	Pem.	C.V.	Mean	S.D.	Pem.	C.V.	Mean	S.D.	Pem.	C.V.	Mean	S.D.	Pem.	C.V.
Liver	3.24	0.41	0.09	12.58	3.27	0.53	0.11	16.28	3.11	0.54	0.10	17.24	3.16	0.87	0.15	27.43
Brain	0.95	0.03	0.01	3.59	0.92	0.03	0.01	3.70	0.94	0.03	0.01	3.51	0.97	0.04	0.01	4.31
Kidneys	0.59	0.04	0.01	7.11	0.61	0.11	0.02	18.81	0.45	0.08	0.02	17.92	0.54	0.06	0.01	11.60
Lungs	0.54	0.06	0.01	10.82	0.48	0.08	0.02	15.94	0.45	0.05	0.01	11.38	0.47	0.07	0.01	15.29
Pancreas	0.37	0.04	0.01	10.11	0.36	0.05	0.01	13.50	0.28	0.04	0.01	14.64	0.36	0.08	0.01	20.99
Heart	0.33	0.03	0.01	7.78	0.34	0.04	0.01	11.08	0.27	0.03	0.01	11.68	0.34	0.04	0.01	10.56
Spleen	0.25	0.07	0.02	28.46	0.25	0.05	0.01	21.95	0.17	0.05	0.01	31.21	0.26	0.05	0.01	20.31
Cord	0.23	0.01	0.00	3.42	0.25	0.01	0.00	4.40	0.25	0.01	0.00	4.82	0.26	0.01	0.00	4.69
Fem. wt	0.22	0.02	0.01	10.19	0.24	0.03	0.01	14.17	0.22	0.02	0.00	10.70	0.23	0.03	0.01	14.67
Uterus	0.21	0.08	0.02	36.62	0.26	0.10	0.02	39.92	0.13	0.07	0.01	51.88	0.18	0.10	0.02	53.63
Submax	0.16	0.02	0.00	12.20	0.17	0.02	0.00	10.53	0.15	0.03	0.01	19.08	0.21	0.05	0.01	24.88
Eyes	0.14	0.01	0.00	9.09	0.15	0.01	0.00	3.45	0.15	0.01	0.00	6.08	0.15	0.01	0.00	6.08
Thymus	0.14	0.04	0.01	30.00	0.14	0.03	0.01	24.26	0.08	0.04	0.01	48.00	0.10	0.01	0.00	10.10
Hum. wt	0.10	0.01	0.00	7.14	0.11	0.01	0.00	11.82	0.09	0.01	0.00	8.51	0.10	0.02	0.00	14.85
Ovaries	0.048	0.009	0.002	18.16	0.048	0.012	0.002	25.05	0.034	0.012	0.002	33.82	0.041	0.013	0.002	32.34
Adrenals	0.021	0.005	0.001	21.63	0.022	0.004	0.001	18.43	0.014	0.003	0.001	18.38	0.018	0.001	0.000	6.70
Thyroid	0.012	0.003	0.001	27.59	0.009	0.001	0.000	8.89					0.007	0.001	0.000	15.49
Hypophysis	0.0041	0.0006	0.0001	14.63	0.0054	0.0009	0.0002	16.67	0.0051	0.0007	0.0002	13.73	0.0047	0.0009	0.0002	19.15
BL/BW	77.6	5.0	1.1	6.38	92.7	10.6	2.2	11.43	74.0	11.7	2.2	15.81	80.7	9.4	1.6	11.65
BW/TL	86.2	2.7	0.6	3.14	86.0	2.2	0.4	2.55	89.0	1.7	0.3	1.91	88.6	3.0	0.5	3.39
BW/HL	12.69	0.27	0.06	2.13	12.75	0.47	0.10	3.69	12.74	0.24	0.04	1.88	12.87	0.30	0.05	2.33
BW/FL	16.40	0.48	0.10	2.93	16.31	0.49	0.10	3.00	16.44	0.36	0.07	2.19	16.58	0.42	0.07	2.53

A comparison of the coefficient of variability of the grams organ weight per 100 grams of body weight and the grams organ weight per 100 millimeters of body length brings out certain consistent differences.

If the variability of these relations is taken as an index of the closeness to which any given structure follows the body weight or the body length as it differs in value among the animals of any particular group, it follows that any organ which shows a consistently marked lower variability in grams per 100 millimeters of body length than in grams per 100 grams of body weight, is probably more closely associated, in its size development, with the factors conditioning growth in body length than it is with the factors of growth in total body weight,—and conversely similarly.

The data show that in animals of the same age the weight of the brain, spinal cord and eyeballs, is correlated with body length rather than with body weight. The growth of the central nervous system is thus shown to be intimately related to the growth of the enveloping skeletal parts. Which is the dominant factor in the association is not easily discernible.

The length of the tail, the humerus and the femur is also correlated with body length rather than with body weight. This indicates a common determining factor in the regulation of the growth in length of the various parts of the skeletal system included in this study and may presumably be extended to the system as a whole.

On the other hand, the weight of the humerus and femur is correlated with body weight rather than with body length. If bone weight is associated with compactness and hence supporting strength it might be expected that this correlation would exist.

The liver, kidneys, spleen, thymus, epididymis, uterus, ovaries and hypophysis show a fairly consistent correlation with body weight, though the differences between the coefficients of the two series are not as great as those exhibited by the structures previously discussed. The relatively greater closeness of fit of the fluctuations of these organs to those of the body weight is probably due to sensitiveness to differences in metabolic adaptation.

The factors concerned in the variability of any given structure within a group of individuals are many and largely indeterminate. Nevertheless, the existence of reasonably consistent trends of organ variability extending through several groups or from group to group allow the formulation of generalizations of biological interest.

In table 5 are given the coefficients of variability of the individual structures in the eight groups of rats. A study of these values with those given in the other tabulations in this paper shows that there is no relation between the differences in size of the various structures and the differences in their variability coefficients, nor between their variability and the growth capacity during the growth period from 100 to 150 days.

The thyroid, thymus, spleen and epididymis in the males, and the uterus, ovaries, thymus, spleen and adrenals in the females tend to show a greater variability than the other organs. They are thus seen to be more sensitive to environmental differences presumably of metabolic origin. In both sexes the eyeballs, brain, body length, humerus length, femur length and tail length are the least variable.

TABLE 5

The variability coefficients

	MALES				FEMALES			
	Ref. control	Control	Thypar	Parathy	Ref. control	Control	Thypar	Parathy
Thyroid	30.83	32.04		40.22	27.32	11.11		21.43
Thymus	26.01	23.73	35.36	26.25	30.40	26.67	44.23	33.73
Epididymis	23.40	14.80	25.57	28.03				
Uterus					37.03	40.70	16.87	63.34
Submax	20.95	10.80	15.40	24.92	12.04	12.09	22.30	26.90
Spleen	17.30	21.08	19.95	34.78	25.84	23.93	33.54	24.93
Femur wt	15.90	11.55	16.16	18.51	11.05	16.40	14.71	20.14
Kidneys	15.41	15.57	19.08	30.38	7.64	20.87	20.33	16.33
Humerus wt	14.73	10.72	14.48	15.47	8.31	14.74	12.91	20.47
Testes	14.46	15.02	20.46	17.48				
Ovaries					18.47	26.39	10.76	34.67
Body wt	13.92	11.44	20.11	17.37	7.63	11.98	17.35	17.00
Hypophysis	13.56	9.72	19.51	15.87	13.89	18.27	16.48	22.89
Lungs	13.02	31.86	19.31	9.13	11.98	42.46	14.18	31.61
Liver	12.97	17.77	21.03	21.45	12.59	17.86	19.11	26.15
Adrenals	12.78	14.40	12.50	12.62	22.28	19.76	19.58	25.63
Heart	12.39	8.39	13.46	14.45	8.76	12.75	16.91	16.06
Pancreas	11.61	13.24	17.55	16.12	10.13	14.94	14.99	24.86
Spinal cord	7.28	4.84	6.24	7.13	4.63	6.51	8.66	9.21
Tail length	6.03	4.06	7.28	6.35	3.88	3.79	3.83	6.55
Fem. length	5.45	3.60	5.78	6.19	3.56	5.58	5.85	6.80
Hum. length	4.50	3.76	4.92	5.48	3.21	3.55	5.36	6.47
Brain	4.35	3.16	4.49	4.43	3.80	4.75	6.04	5.62
Body length	4.32	2.62	5.65	5.00	2.30	3.42	4.37	5.97
Eyeballs	3.56	2.86	3.04	3.25	10.23	2.76	5.41	3.20

At 100 days of age the general variability of the males is greater than that of the females. At 150 days of age the females are the more variable in the control and the parathy groups. In the thypar groups no consistent sex difference obtains. This greater variability in the adult female may be due to the physiological response to the ovulation cycle.

These observations are in fair agreement with those of Jackson (3) on the normal variability of the albino rat.

The variability of the different organs is fairly consistently greater in the thyroidless and parathyroidless rats than in the controls. The metabolic imbalance has produced a chaotic response which is mirrored by the general increase in organ variability. The effect is less marked in the thypars, probably because of the lowering of the plane of general metabolism. This is particularly noticeable in the females.

We come now to the discussion of the growth capacities.

During the period from 100 to 150 days in the life of the normal albino rat there occurs an increase in weight and length of the animal. All of the organs participate in the weight increase, and the tail, the humerus and femur increase in length. The amount of this increase expressed in per cent of the original size (grams per 100 grams or millimeters per 100 millimeters) is a measure of the intensity of the growth property. It is called the growth capacity (G.C.).

Table 6 contains the figures for the G.C. of the various structures of the male and female rats of the three groups arranged in the order of decreasing magnitude in the male controls.

A rough comparison of the male and female controls brings out certain similarities in the serial disposition of the G.C. of the 23 measurements. In both sexes the G.C. of the thyroid, body weight and femur weight is high with respect to that of the other parts and in a group comprising the top third. In addition it should be noted that the epididymis and the uterus as representatives of the reproductive system apart from the gonads also fall into this first group. The submaxillary, heart, spinal cord and liver are found in the middle third; while the bottom bracket contains the gonads, the thymus, eyeballs, lungs and brain. In both sexes the G.C. of the tail in length is less than that of the body, humerus and femur, the three last making a separate group.

These groupings indicate a certain similarity of trend in the differential development of the two sexes. This similarity is probably generic. On the other hand identity, both in absolute and in relative G.C., is lacking.

With the exception of the hypophysis and the adrenals the G.C. of all the structures measured is greater in the males than in the females. This of itself would not be demonstration of a sex difference in differential development (with the exception of the two organs noted above), for proportional differential development can be conceived of, in which the degree of superiority of the males is the same for each individual organ. If, however, there is no quantitative sex-uniformity of difference between the G.C. of the various organs, differential development is not quantitatively the same in both sexes.

In table 7 are given the value for the G.C. of the several structures in the females in terms of those of the males. It is quite evident that the G.C. in the females is not a uniform percentage of that in the males.

Hence quantitative differential development is not the same in the females as it is in the males.

Of special interest is the fact that the members of a given system, such as the nervous system, represented by the brain, spinal cord and eyeballs, the skeletal system, represented by the humerus and femur (weight), and the reproductive system (ovaries-testes; uterus-epididymis) show as systems a uniform per cent sex difference in G.C. That is to say,

TABLE 6

The G.C. of the various organs in the male and female rats of the three groups

	MALES			FEMALES		
	Controls	Thypars	Parathys*	Controls	Thypars	Parathys
Epidid. (uterus)	73.8	48.0	77.1	35.0	−35.6	−10.9
Body wt	40.3	12.5	23.0	25.1	−7.1	5.2
Kidneys	38.8	−15.2	13.1	20.4	−16.9	4.8
Thyroid	34.6		22.1	29.8		15.0
Fem. wt	32.7	11.0	21.9	24.0	2.8	10.3
Spleen	32.0	−12.2	44.5	18.5	−22.4	23.7
Hum. wt	31.6	8.8	18.8	24.6	−0.7	9.7
Submax	31.6	0.2	41.2	21.1	0.1	44.2
Hypophysis	28.6	46.4	16.7	60.0	40.0	36.1
Heart	27.3	−7.3	21.0	19.2	−9.3	15.0
Pancreas	25.1	−10.5	21.7	14.7	−18.3	10.7
Cord	23.8	12.4	21.6	20.1	10.8	17.6
Liver	23.0	−10.0	14.5	17.8	3.2	10.2
Testes (ovaries)	21.7	11.1	23.7	11.3	−26.7	−10.2
Thymus	20.5	−32.5	−9.5	8.0	−44.5	−25.7
Lungs	19.5	−6.6	7.7	6.9	−9.6	16.2
Eyes	15.7	14.1	15.8	13.9	7.0	10.5
Adrenals	10.5	−12.3	1.9	23.1	−28.8	−0.9
Brain	7.2	1.5	7.8	5.9	0.2	5.3
Fem. L	13.7	5.8	10.1	10.3	2.8	6.1
Hum. L	12.8	5.4	9.0	11.0	2.8	6.0
Body L	12.5	4.4	8.0	11.0	2.7	5.2
Tail L	8.5	3.2	5.5	6.7	1.3	3.3

* Corrected.

the differential development of the various parts of the central nervous system, the reproductive system and the skeletel system in weight is sex proportionate.

Turning now to the thypars, it is seen from table 6 that the lack of thyroid secretion has resulted in a general decrease in the G.C. with the exception of the hypophysis in the males. The question that at once arises is: has this lowering of the plane of development been proportionate or disproportionate?

Since in some cases development of the individual organ in both sexes had been not only retarded, but reversed and retrogression brought about in which an actual loss of weight has occurred, while in others some growth has taken place, it is clear that thyroid lack causes disproportionate differential development. The direction and extent of this are given in table 8.

TABLE 7

The G.C. of the various organs in the females in terms of that of the males

$$\frac{\text{♂ G.C.}}{\text{♀ G.C.}}$$

CONTROLS		THYPARS		PARATHYS	
Organ	Relative G.C.	Organ	Relative G.C.	Organ	Relative G.C.
Adrenals	220	Liver	−344♂	Hypophysis	216
Hypophysis	210	Kidneys	90	Lungs	210
Eyes	89	Cord	87	Submax	107
Body L	88	Hypophysis	86	Cord	82
Hum. L	86	Heart	79	Spleen	74
Thyroid	86	Thymus	73	Heart	71
Cord	84	Lungs	69	Liver	70
Brain	82	Body L	61	Brain	68
Tail L	78	Thyroid		Thyroid	68
Hum. wt	78	Pancreas	57	Eyes	67
Liver	77	Spleen	54	Hum. L	67
Fem. L	75	Hum. L	52	Body L	65
Fem. wt	73	Eyes	50	Fem. L	60
Heart	70	Submax	50	Tail L	60
Submax	67	Fem. L	48	Hum. wt	52
Body wt	62	Adrenals	43	Pancreas	49
Pancreas	59	Tail L	41	Fem. wt	47
Spleen	58	Fem. wt	39	Kidneys	37
Kidneys	53	Brain	1	Thymus	37
Ovaries (testes)	52	Hum. wt	−8	Body wt	23
Uterus (epidid.)	47	Body wt	−57	Uterus (epidid.)	−14
Thymus	39	Uterus (epidid.)	−74	Ovaries (testes)	−43
Lungs	35	Ovaries (testes)	−241	Adrenals	−47

Two facts emerge from this tabulation. The first is that the extent of the disturbance of development bears no relation to the normal G.C. as far as an inter-organ comparison is concerned. The second is that the organs primarily concerned with the vegetative functions of the body are the ones that suffer the most. The disturbance of development is greater in these organs in both sexes than is that of the body as a whole or of its other parts. Such retrogression implies a disturbance in functional effectiveness. It is significant of a profound distortion of the fundamental

mechanisms concerned in the assimilation, transport and excretion of ingested metabolites.

These facts lead to the conclusion that a cause of the general retardation of growth in thyroidless animals lies in the peculiar sensitiveness of the organs of vegetative function to thyroid deficiency with the concomitant inadequateness of the preparation for utilization of the ingested foodstuffs. I do not desire to give the impression that the slowing of development is

TABLE 8

The relative G.C. of the various organs of the thypar and parathy groups in terms of the controls

THYPARS			PARATHYS		
Organ	Males*	Females	Organ	Males	Females
Hypophysis	162.2	66.7	Spleen	139.1	128.1
Eyes	82.5	50.4	Submax	130.4	209.4
Epidid. (uterus)	65.0	−107.7	Testes (ovaries)	109.2	−90.3
Cord	51.9	53.7	Brain	108.3	89.8
Testes (ovaries)	51.2	−236.3	Eyes	100.6	75.5
Fem. wt	33.6	11.7	Epidid. (uterus)	97.2	−31.1
Body wt	31.0	−28.2	Cord	90.8	87.6
Hum. wt	27.8	−2.8	Pancreas	86.5	72.8
Brain	20.5	3.4	Heart	76.9	78.2
Submax	0.6	0.5	Fem. wt	67.0	42.9
Lungs	−21.9	−47.8	Thyroid	63.9	47.3
Heart	−26.7	−48.4	Liver	63.0	57.3
Spleen	−38.1	−121.1	Hum. wt	59.5	39.4
Kidneys	−39.1	−82.8	Hypophysis	58.4	60.2
Pancreas	−42.0	−124.5	Body wt	57.1	20.7
Liver	−43.5	18.0	Kidneys	33.8	23.5
Thyroid			Lungs	25.7	80.6
Adrenal	−123.0	−124.7	Adrenals	18.1	−3.9
Thymus	−157.2	−556.2	Thymus	−46.3	−321.2
Fem. L.	42.3	27.2	Fem. L.	73.7	59.2
Hum. L.	42.2	25.4	Hum. L.	70.3	54.5
Body L.	35.2	24.5	Body L.	64.0	47.2
Tail L.	37.2	19.4	Tail L.	63.9	49.3

* Corrected.

due entirely to the laying down on the job of the lungs, heart, liver, kidneys, spleen and pancreas, or that a generalized lowering of metabolic processes is not caused by thyroid deficiency. It does seem to me, however, that since these organs are apparently more dependent upon thyroid secretion for their own development than are other parts of the body, and since they are the main mechanisms for the making available of the essentials of growth, that their probable loss of functioning effectiveness is a major contributing factor in the retardation of general growth observed under these and similar experimental conditions.

The significance of the dependency of these organs on thyroid function is also important in an understanding of the pathogenesis of hypothyroid conditions. The throwing out of gear of a whole series of organs such as this cannot help but have a profound and disturbing influence on intermediary metabolism entirely aside from any specific effect of thyroid secretion lack on general cell metabolism. The one may well restrict and change the nature of the materials offered to the tissues for utilization, the other lower the ability for utilization of what is offered. The etiology, then, of the patholgy of hypothyroid states does not lie entirely in the domain of a simple lowering of basal metabolism, but includes and importantly, the disturbances of intermediary metabolism mediated by the vegetative organs of preparation, assimilation, transport and excretion.

The values recorded in the tables show that the reduction in G.C. due to thyroid (thypar) lack is greater in the females than in the males in every case save the liver. From table 7 information of particular interest is derived. In the first place the distortion of differential development is shown to differ in the two sexes both qualitatively and quantitatively. In the second place the systemic coördination of differential development observed in the controls in not maintained after thyro-parathyroidectomy.

Turning now to the parathys it is seen that the toxemia induced by the lack of parathyroid function causes a distortion of differential development in both sexes. A sex difference is shown and a difference from that produced by thyroid (thypar) deficiency both qualitatively and quantitatively. The extent of the decrease in the G.C. of the individual structures is constantly less in the parathys than in the thypars and less in the males than in the females with one or two exceptions. The percentage decrease of the G.C. in the parathys as compared with that in the thypars is not of uniform value, and in two cases where decreases occurred after thyro-parathyroidectomy, acceleration occurred after parathyroidectomy (spleen and submaxillary glands).

Certain general similarities in reduction in G.C. are, however, exhibited in both groups. The thymus, adrenals and kidneys of both sexes are quite a bit more sensitive than the other structures to both types of incretory deprivation. This may mean that their response in the thypars is largely due to parathyroid loss, or it may mean that these organs are fundamentally more labile. Similar relative intensity of response is shown by the ovaries and uterus in both groups. Jackson (4) has reported that the ovaries and thymus are quite sensitive to metabolic derangements produced by inadequate diets, and hence the latter interpretation has at the present the more favorable aspect for these organs at least.

In the thypars the G.C. of the spinal cord, heart, pancreas and spleen of the females is practically the same per cent of the G.C. of these organs

in the males as is found in the controls. Hence, the distortion of the differential development of these organs caused by thyroid deficiency shows no sex difference. The same holds true for the hypophysis, spinal cord, liver, heart and thymus in the parathys. A notable fact about this stability in sex relationship is that the spinal cord and heart growth in the female maintains its relation to the growth in the male under the conditions of thyroid (thypar) deficiency and parathyroid toxemia, while the sex relations of the other organs are greatly distorted.

Finally there is evident in the parathys a tendency for the maintenance of the systemic coördinated development present in the controls. This tendency is not brilliantly successful, but incoördination is not sufficiently intensive to bring the members of the system, particularly humerus and femur, so very far apart.

A study of the tables given in this paper reveals that there is no correlation between the size of a given organ and its G.C. as compared with the size and G.C. of the other organs. Furthermore, on the same basis there is no correlation between the normal G.C. and the reduction in G.C. caused by the thyroid (thypar) or parathyroid deficiency. It must be held in mind, however, that we are here dealing with but a single panel in the picture of growth and that until records are had of the course of growth in the preceding periods interpretation is incomplete. Nevertheless certain principles of development are discernible.

The anatomical development of a type of organism characteristic of a given species is the result of the inherited specific factors. Such development is qualitative. The limitations of the extent of development are also a matter of heredity, but the variations in development within the limits are conditioned by the potential value of the intensity of the growth impulse and the degree to which the environment permits or prohibits its expression. It is only under optimal conditions that full expression of the growth impulse is to be found. I doubt that it is ever attained. Such is the quantitative aspect of development.

In the development of a multi-organic animal such as the rat or man, the relative rate of growth of the various parts is a necessary consequence of the relative strength of the factors which determine what part of the whole the individual structure is to take plus environmental influences. As a result of the prime directing agencies under conditions of uniform environment the animal at any given stage of its development is a composite of structures, each of which tends to bear a specific size relationship to the organism as a whole and to the other parts. Various environmental influences, however, may and do modify interrelationships of development. Hence, although the limits of quantitative differential development within the organism can be considered as being fixed by the specific inherited factors, the actual relative extent of inter-

organ development may show variations within limits caused by environmental conditions.

The relative size of the various organs is furthermore determined by the extent to which growth by increase in cell number and growth by increase in cell size can proceed. Such growth is conditioned by the inherent specific differences in type metabolism. Therefore, since the different types of metabolism must exert different effects on the capacity for growth as expressed in cell reproduction and assimilation, it is obvious that relative size and relative G.C. can show no consistent relationships in maturely functioning organisms. We are thus brought to associate growth capacity with type metabolism. It should be kept in mind that we are comparing the relation between the size and G.C. of different organs, not the relation between the size and G.C. of an individual structure. Normal, specific, proportionate, differential development results when the activity and interactivity in kind and degree of the type metabolism of the various organs is harmonious and natural. When this harmony is disturbed disproportionate differential development may result.

Such a disturbance in environment is caused by the loss of the thyroid and parathyroid secretions. Distortion of differential development is caused by the ensuing metabolic imbalance. The milieu of reaction is altered and the effect on the G.C. of such alteration is associated with the kind and amount of disturbance of the specific type metabolism of the individual organ with its rebound on cell assimilations and cell reproduction rather than with the normal intensity of the G.C. as such. To this disharmony all contribute.

The results of this study and the foregoing derivatives hold for these young, sexually mature, growing animals. During embryonic development when type metabolism is at low ebb, it is possible that Stockard's (5) belief—"when we slow the rate of development we affect that part which chances to be developing at its maximum rate at that time, while the more slowly developing parts are not so seriously injured if at all"—is a correct expression of the state of affairs.

Stockard (6) has recently discussed the probable rôle of thyroid activity in the development of structural types. My results show that the lack of thyroid (thypar) secretion for a 50-day interval in the life of the albino rat after sexual maturity has been reached, has practically no influence on the relative length of the long bones with respect to body length, but that the animals become less heavy for their length. Since this also holds for the parathys it is apparently not a specific thyroid effect. It is, therefore, evident that the lack of the thyroid apparatus in rats at this age does not produce a specific hypothyroid type of external structure. Nevertheless, what has already been written does show definitely that a rearrangement of organic proportion is caused by thyroid deprivation

which is different from that caused by parathyroid loss, and it is possible that such disturbance if initiated at an earlier developmental stage and carried on for a longer period might result in a definite structural type characteristic of thyroid deficiency. Studies of this phase of the subject are now under way.

Although there is no detectable relation between the size of the different organs and the G.C. values there is a distinct relation between the size of any given organ and its G.C. Since the number of individual observations for any single organ is at present too small to allow the establishment of a satisfactory mathematical expression of the relationship, it would be waste of space to give the large number of tables necessary for complete demonstration. For the present, then, I will confine myself to a brief statement of the correlation and reserve the analysis for a later occasion.

In general those rats which are below the group mean in body weight at 100 days of age tend to have a greater G.C. for the ensuing 50 days than do those rats which are above the mean. That is to say, G.C. and body weight tend to be negatively correlated. This relation I showed to exist in the growth of new-born infants the first two weeks after birth (7). Those babies which were the lightest weight at birth grew the more rapidly, those which were the heaviest grew the more slowly.

This relationship between body weight and G.C. is shown in the thypar and parathy groups as in the controls, and extends to the individual structures included in this study.

The reduction of the G.C. caused by the lack of thyroid and parathyroid function tends to be negatively correlated with the normal expected G.C. of the individual organ and positively correlated with its size. To be concrete,—the G.C. of the heart which is below the group mean in weight at 100 days of age and which accordingly tends to have a greater G.C. than the mean G.C. for all hearts of the group, tends to be reduced by a lesser degree than is the G.C. of a heart which is above the group mean in weight and which tends to have a lesser G.C. than the mean G.C. for all the hearts of the group.

SUMMARY AND CONCLUSION

The differential development of the normal, female, sexually mature albino rat between the ages of 100 and 150 days is similar to but not identical with that of the male.

When the thyroid apparatus is removed disproportionate differential development is produced in both sexes, similar but not identical for each. The disturbance is quantitatively greater in the females and is in the direction of a general lowering of the growth capacity.

Those organs which are concerned in the vegetative functions of the body are in both sexes more seriously distorted in development than are the other parts of the organism. The lungs, heart, kidneys, spleen, liver, adrenals, pancreas and thymus not only cease their growth but actually lose weight. This is taken to indicate that the general retardation of growth observed in athyroid conditions is as much due to an inadequate preparation and presentation of metabolites to the tissues as to a general lowering of cell metabolism. The significance of this in the etiology of the pathology of hypothyroid states is pointed out.

The toxemia of parathyroid deficiency also causes a distortion of differential development which differs in the two sexes, but is not as great in degree or the same in kind as that which occurs following thyroparathyroidectomy.

Notwithstanding the fact that the absolute weight of the body and its parts is greater at the given ages in the males than in the females, with the exception of the hypophysis and the adrenals, all the organs, save the liver, are a greater proportion of the total body weight in the females than in the males. This difference may be attributed to the difference in reproductive mechanism of the two sexes.

The weight of the brain, spinal cord and eyeballs, and the length of the humerus, femur and tail are more closely associated with body length than with body weight. Which is the dominant factor in the association of the parts of the nervous system studied with the enveloping skeletal parts is not clear.

The liver, kidneys, spleen, thymus, epididymis, uterus and ovaries show a fairly consistent correlation with body weight rather than with body length. This may be attributed to a sensitiveness to differences in metabolic adaptation.

The thyroid, thymus, spleen and epididymis in the males, and the uterus, ovaries, thymus, spleen and adrenals in the females tend to be highly variable. In both sexes the brain, eyeballs, body length, tail length, and humerus and femur length are least variable of the 23 structures measured.

There is no correlation between the differences in size of the various parts and the differences in their variability coefficients.

There is no correlation between the differences in size of the various organs and their difference in growth capacity, or between the differences in growth capacity and the differences in variability.

The variability of the various organs in the rats of the thypar and parathy groups tends to be greater than that of the controls. This is due to metabolic imbalance caused by the lack of incretory products.

The growth capacity of an individual organ is negatively correlated with its initial size. This holds in all groups. The reduction of growth

capacity due to the lack of the incretory products is positively correlated with the initial size of the individual organ and negatively correlated with its normal expected growth capacity.

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PARABIOSIS IN THE STUDY OF DEFICIENCY DISEASES

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The existence of chemical entities in foodstuff, called vitamines, and their relation to certain dietary deficiency diseases, may be regarded as definitely established. The number of these substances, their chemical nature, and of perhaps greatest importance, the relation which these substances bear to the disorders of metabolism attributed to their absence, are for the most part obscure. It cannot be said that the pathogenesis of any of the deficiency diseases in man has been completely demonstrated. It can be easily shown that synthetic diets deficient in one or more of the vitamines lead to failure in normal growth and nutrition in appropriate animals and that definite pathological changes often arise which closely resemble those seen in the so-called deficiency diseases in man. It is not however clear how the lack of a specific element in the diet can by itself lead to the production of pathologic changes, often of inflammatory character, which are commonly associated only with the action of irritants. The pathology of the diseases in man is not that of starvation, as one might expect according to this view of the pathogenesis.

While it is true that the absence of one or more vitamines from the diet leads to a definite and characteristic disturbance, and in this sense the lack of the vitamine is the etiologic agent, still the pathogenesis of the disturbance may be far more complex. It is quite possible that the outstanding pathological changes in many of these diseases are due to the invasion by microörganisms of certain tissues that have lost their normal resistance due to the vitamine deficiency. This appears to be the case in xerophthalmia. Again it is possible that the vitamine deficiency may bring about some disturbance in metabolism so that poisonous materials are produced or accumulate in the body and cause disease. In this connection it is well to consider the alimentary tract as a source for such intoxication since it is well established that dietary changes may alter the production of intestinal poisons and there is evidence that in some deficiency diseases (scurvy, pellagra and beri-beri) alteration in the permeability of the intestinal mucosa leading to increased absorption of these toxic products may be present. The latter view is in harmony with the observation (both experimental and clinical) that when the deficiency disease becomes well advanced

it is often impossible to check its further progress by any treatment, dietetic or medicinal. This fact is difficult to reconcile with our ordinary concepts of partial starvation.

In order to test the possibility that a deficiency disease is in reality a toxemia secondary to a specific vitamine deficiency, experiments were planned using experimental animals, joined together in pairs, or in parabiosis. Georg Schmidt (1) in a recent exhaustive review of the literature on parabiosis has pointed out the usefulness of this procedure in studying many biologic problems ranging from endocrinology to experimental ileus. The advantage of the method lies in the fact that when such animals are living together harmoniously, certain of the body constituents of one animal may pass over to the other. There is no direct vascular continuity between the animals and substances in the blood stream must pass from one animal to the other by diffusion into the abdominal cavity, which is common to both, and subsequent reabsorption. Forsbach (2) tried to get evidence of the internal secretion of the pancreas by this method and found apparently that it could pass from one animal to the other. It has subsequently been found that many substances when injected into one animal may appear in the blood and urine of the second. This is true in the case of potassium iodide, lactose, phenolsulphonephthalein, strychnine, methylene blue, trypan blue, and a number of other substances.

Accordingly it occurred to us that if the vitamine deficiencies gave rise to a toxemia, if such a deficiency was induced in one of such animals, the other might also display similar symptoms. Attempts to produce parabiotic chickens for the study of vitamine B were unsuccessful because of the great technical difficulty in the operation. Guinea pigs were however successfully united for the study of vitamine C, but the animals were so susceptible to various infections and other influences that no accurate results have so far been obtained. White rats, however, serve admirably for the study of vitamine A, and after the technic of the procedure has been mastered they may be united and kept living harmoniously together in good condition for months. We have kept several pairs alive for two and three months and in each case death occurred through accident. A male and a female, or two males, or two females, may live together in this fashion. It should be noted however that many parabiotic pairs die suddenly from some cause at present inknown. There are no definite post-mortem abnormalities. In other cases, particularly when there is a marked discrepancy in size or strength between the animals at operation, the smaller may fail to grow, become weak and anemic and die in the course of three or four weeks. The great majority of animals die from some accident or intercurrent infection.

In the following experiments young white rats from the same litter were united with the usual technic which in many respects resembles

that of gastro-enterostomy. The animals were anesthetized with ether and tied side to side on the operating table. An incision was made through the skin in the mid-clavicular line extending the entire length of the abdomen from the costal margin to the inguinal ligament. The adjoining skin edges in each animal were then united bringing the raw edges together. A second line of sutures then fixed the abdominal muscles of one animal to the other. Following this the abdominal cavities of both were opened and a transposition of the intestines effected. The remaining layers were then closed so that the animals had a common abdominal cavity. No bandages were used but the adjacent legs of both animals were fixed together with adhesive tape so that they could not pull apart. Even with this precaution there was always considerable tension on the uniting band and in several cases it was stretched out 7 or 8 cm.

The special diet lacking in vitamine A was prepared as follows:

Casein, 20 per cent (this was specially prepared and extracted with alcohol and ether until free of fat)

Inorganic salts, 4 per cent (the mixture recommended by McCollum, and believed to contain all the mineral essentials was used)

Yeast extract (vitamine B), 5 per cent (an extract made from Fleischmann's starch-free yeast)

Crisco, 25 per cent (this is a hydrogenated cotton seed oil. The fat was kept melted and aerated for 4 hours)

Cornstarch, 46 per cent (Kingsford's cornstarch, untreated)

Purified agar-agar, 4 per cent (the agar was dissolved in distilled water, and the other articles of the diet stirred into the mixture. A fresh diet was made up every second day)

The absence of vitamine A from the diet described above was determined by the following biological test. Young white rats, weighing between 40 and 50 grams, were placed on a synthetic diet known to have a high content of vitamine A. This diet was exactly similar to the vitamine-free diet except that butter fat and cod liver oil were given instead of the Crisco. The animals soon showed a distinct gain in weight, usually 10 grams or more in two weeks. They were then placed on the test diet, which contained no vitamine A. The absence of the vitamine was indicated by the cessation in growth or decrease in weight, the development of a rough thin hair, and other symptoms (irritability and hyperexcitability) which we noted in other rats on diets lacking in vitamine A.

EXPERIMENTAL PROCEDURE. *Experiment 1.* April 15, 1921. Parabiosis operation, with a male and female white rat.

Female, weight	47 grams
Male, weight	49 grams
Total	96 grams

April 15–23. Diet of milk, bread, lettuce and grain. Condition good. Total weight 98 grams.

April 24. Condition good. Weight combined 98 grams. Both placed on a diet lacking in vitamine A. The male was given cod liver oil daily, ½ gram (vitamine A).

April 25–May 1. There has been a gradual loss in weight and strength particularly noticeable in the female. Combined weight 74 grams. The contrast between the male which received the vitamine and the female was marked, and it was easily apparent that the loss in weight noted was almost wholly due to the failure of growth of the female.

May 2, 1921. Both animals given milk, bread, lettuce and grain.

May 3–6. Steady and marked improvement.

May 7. Condition of both good. Combined weight 98 grams.

May 8. The male was accidentally killed in the cage. Female was sacrificed. Combined weight after death 97 grams. At autopsy there was found a complete transposition of intestines, those of the male being anchored firmly by adhesions in the abdominal cavity of the female and those of the female in the abdomen of the male.

Experiment 2. November 10, 1921. Parabiosis operation, with a male and female white rat. Rats were 30 days old.

Male weight	44 grams
Female weight	38 grams
Total	82 grams

November 10–14. Condition good. Diet of bread, milk, lettuce, and grain.

November 14. Combined weight 87 grams. Both animals were placed on the special diet lacking vitamine A. The female was given ½ gram of cod liver oil daily.

November 18. Combined weight 80 grams.

November 21. Combined weight 82 grams. The male is definitely smaller and weaker than the female.

November 24. Combined weight 77 grams. The male is very thin and anemic, is much weaker and smaller than the female.

November 25. The male was given ½ gram of cod liver oil daily and the female ½ gram of lard.

November 29. Combined weight 77 grams. The male is still thin and weak.

November 30. Combined weight 75 grams.

December 1. The male was given 2 grams of butter. The female appears to be getting weaker.

December 3. The male has definitely improved. The female is getting gradually weaker.

December 5. The male was accidentally killed, the female dying 6 hours later. Post-mortem examination showed the usual transposition of intestines. The animals were separated and weighed: male weight, 33 grams; female weight, 36 grams.

Experiment 3. November 17, 1921. Parabiosis operation, with a male and female white rat. Rats were 19 days old.

Male weight	33 grams
Female weight	31 grams
Combined weight	64 grams

November 18–23. Diet of milk, bread, lettuce and grain.

November 23. Condition good. Combined weight 68 grams. Both animals were placed on a special diet lacking in vitamine A. The male was given ½ gram of cod liver oil daily and the female ½ gram of lard.

November 24. Combined weight 67 grams. Condition good.

November 25–December 7. The female has stopped growing and is much weaker and smaller. The male is larger and in good condition. Combined weight 66 grams. (See fig. 1.)

December 8. Both animals were placed on a diet of milk, bread and lettuce and grain. A water solution of Trypan blue was injected subcutaneously in the male and in a few hours he became deeply colored throughout. Six hours later the female became colored over the abdomen and in 48 hours both animals were equally colored by the dye.

December 11. Condition good. Combined weight 100 grams.
December 14. Condition good. Combined weight 116 grams.
December 23. Condition good. Combined weight 167 grams.
December 26. Condition good. Combined weight 185 grams.

Fig. 1

Fig. 2

Fig. 1. Picture taken December 7, 1921. The parabiotic twins had been on the special vitamine A free diet for 14 days. The rat on the right was given ½ gram of cod liver oil daily and its growth was normal. The failure of growth on the part of the other twin indicates that vitamine A does not pass from one to the other of such animals.

Fig. 2. Picture taken January 19, 1922. The rat on the right has not received any vitamine A for 18 days, whereas the rat on the left has been given abundant cod liver oil during this period. The difference in size is apparent. These are the same animals shown in figure 1. The reversal in vitamine feeding has caused a corresponding reversal in their relative size.

January 1, 1922. Condition good. Combined weight 202 grams.

The male is larger than the female. Both animals were placed on the special diet lacking in vitamine A. This time the female was given ½ gram of cod liver oil daily and the male ½ gram of lard.

January 8. Condition good. Combined weight 186 grams.

January 9. Combined weight 182 grams. The female is larger and stronger than the male.

January 10. Combined weight 178 grams. The male is definitely thinner and weaker than the female.

January 12. Combined weight 170 grams.

January 18. Combined weight 152 grams.

January 19. Combined weight 150 grams. The male is very thin, vertebrae prominent, and has developed a profuse diarrhea. The female is thinner than on January 1, but is strong, active and well nourished. (See fig. 2.) Both animals were placed on a diet of milk, bread, lettuce and grain.

January 24. Condition much improved. Combined weight 200 grams. The animals were then cut apart and the incisions closed. Female weight, 110 grams; male weight, 90 grams.

Fig. 3

Fig. 4

Fig. 3. Picture taken August 1, 1922. These parabiotic rats have been living harmoniously together 90 days. When the operation was done both were of the same size. Although kept on an adequate stock diet, the female on the right has failed to grow. The male to the left is as large as unoperated control from the same litter. Such stunting in one of a pair of parabiotic twins has been occasionally seen but is unaccounted for.

Fig. 4. Picture taken June 13, 1922. The parabiotic twins had been on the special vitamine A free diet for 9 days. The female on the left was given ½ gram of cod liver oil daily. The male on the right received ½ gram of lard daily during this period. The failure of growth in the male indicates that the vitamine cannot pass from one of such animals to the other.

February 4. Both animals in excellent condition. Female weight, 122 grams; male weight, 130 grams.

Experiment 4. May 1, 1922. Parabiosis operation with a male and female white rat.

Male weight	30 grams
Female weight	28 grams
Combined weight	58 grams

May 2. Both animals were placed on a diet of bread, milk, lettuce and grain

June 1. Condition excellent. Combined weight 80 grams. The male is distinctly larger and stronger than the female.

July 1. Condition good. Combined weight 132 grams. The male is much larger and stronger than the female. The female is in good condition, active, and eats readily.

August 1. Condition good. Combined weight 185 grams. The male is almost twice as large as the female. Both animals are very active, but the female is definitely stunted. There has been practically no growth during the past month. (See fig. 3.)

August 6. The female was accidentally killed. The male died 10 hours later. Post-mortem examination showed the usual transposition of the intestines but no other pathological findings.

Experiment 5. June 1, 1922. Parabiosis operation with a male and a female white rat. Rats were 22 days old.

Male weight	66 grams
Female weight	65 grams
Combined weight	131 grams

June 1–4. Condition good. Diet of bread, milk, lettuce and grain.

June 4. Combined weight 133 grams. Both animals were placed on the special diet lacking in vitamine A. The female was given $\frac{1}{2}$ gram of cod liver oil daily. The male was given $\frac{1}{2}$ gram of lard.

June 11. Condition good. Combined weight 104 grams. The female is distinctly larger and stronger than the male.

June 13. Combined weight 92 grams. The male has become progressively weaker and smaller while the female has increased in size and strength. (See fig. 4.)

June 13. The male was put on a daily ration of $\frac{1}{2}$ gram of cod liver oil, and the female on $\frac{1}{2}$ gram of lard.

June 14. Combined weight 90 grams. The male is still very thin and weak.

June 15. Combined weight 89 grams. The male has not improved but the female has become gradually weaker.

June 16. Both animals were placed on a diet of bread, milk, lettuce and grain.

June 20. Condition of both greatly improved. Combined weight 102 grams.

June 21. The male was accidentally killed. The female lived 12 hours after the death of the male. At post-mortem examination several loops of intestines of the female were anchored firmly in the abdomen of the male.

Experiment 6. June 29, 1922. Parabiosis operation was done, with two male white rats.

Rat 1, weight	60 grams
Rat 2, weight	63 grams
Combined weight	123 grams

June 30. Both animals were placed on a diet of bread, milk, lettuce and grain.

July 6. Condition excellent. Combined weight 132 grams. Both animals were placed on a special diet lacking in vitamine A. Rat 1 was given 1 gram of cod liver oil, and rat 2 was given 1 gram of Crisco (contains no vitamine A) in addition to the special diet.

July 10. Condition good. Combined weight 130 grams.

July 20. Condition good. Rat 2 is perceptibly thinner and weaker than rat 1. Combined weight 128 grams.

July 24. Condition good. Combined weight 118 grams. Rat 2 is definitely thinner and smaller than rat 1, and the vertebrae are more prominent. (See fig. 5.)

July 25. Condition good. Rat 2 is gaining appreciably in weight and strength. Combined weight 116 grams. Animals were continued on the special diet lacking in vitamine A, but rat 1 was now given 1 gram of Crisco and rat 2 was given 1 gram of cod liver oil daily.

July 26–27. Condition good. Rat 2 is gaining appreciably in weight and strength. Combined weight 121 grams.

July 28. Condition good. Combined weight 125 grams. Rat 2 is gaining in weight and strength.

July 29. Rat 1 found dead in cage. Rat 2 was killed immediately. Autopsy revealed the usual transposition of the intestines. A large loop of the intestine of rat 1 was strangulated and necrotic and this was apparently the cause of death.

Fig. 5. Both animals had been given a special diet lacking in vitamine A for 27 days. The rat to the left had received 1 gram of cod liver oil daily, and the rat to the right 1 gram of Crisco daily, in addition to the special diet. The animal fed Crisco was originally the larger of the two. The experiment indicates that vitamine A will not pass from one of such parabiotic twins to the other.

Discussion. The evidence is conclusive that when vitamine A is absent from the diet of one of a pair of parabiotic animals it will manifest the usual signs of such vitamine deficiency even though the mate be given excessive quantities of the vitamine and its growth be normal. The fact may be interpreted in several ways. It seems most probable that there is no intoxication associated with the vitamine absence or both animals should have developed those symptoms eventually. It is possible, though not probable, that toxic products may be present that cannot pass from one animal to the other. The vitamine itself cannot pass from one of these animals to the other, or if so it is unable to perform its function when absorbed from the abdominal cavity. It seems probable that it does not pass across. It is stated (3) that vitamine A is ineffective when given parenterally but this is difficult to reconcile with the fact that the developing embryo in utero must make use of the vitamine supplied in this way. The significance of the failure of vitamine A to pass from one animal to the other with respect to its probable chemical nature will remain obscure until more is known of the selective permeability of the membranes involved. The experiment cannot be interpreted to prove that the molecular size of the vitamine is necessarily large, particularly in view of

the recent interesting experiments of Professor Kahlenberg (4). He has prepared artificial membranes impregnated with various cholesterol esters and similar substances which show a highly selective permeability. Thus he can separate sugars and urea, and even sodium chloride and nickel chloride. The complexity of the situation is evident when it is recalled that vitamine A can apparently pass through not only the placental membranes but it has also been found by one of us (5) in the urine, saliva, gastric and pancreatic juice.

CONCLUSIONS

1. Vitamine A, even if given in great excess to one of a pair of parabiotic twins, does not suffice to prevent the appearance of typical deficiency symptoms in the mate who is not fed any of the vitamine.

2. There is either no toxemia in the early stages of the vitamine A deficiency disorder or, if so, these toxins do not pass to the control parabiotic animal in sufficient amounts to definitely induce the metabolic disturbance.

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I. PREPARATION OF INSULIN

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SEPARATION OF THE TOXIC AND ANTIDIABETIC FRACTIONS. In October, 1922, when a program was outlined by Doctor Carlson to study the different methods of administration of insulin, there was little insulin obtainable by our laboratory for experimental purposes. Only general directions were published as to methods of preparation, so to carry out the work it was necessary to manufacture our own insulin. The method used by the Toronto people, as published at that time, was tried but it was found almost impossible to prepare insulin according to their directions with the equipment then available. After three months, with very little success, we learned at the meetings of the American Physiological Society at Toronto in 1922 that Doctor Shaffer and his co-workers had developed a new method for the isolation of the pancreatic hormone. He kindly furnished us with the most explicit directions, and by following them faithfully we were able to prepare an active product. In the course of the work we modified and believe greatly improved the fine method proposed by the above workers. A brief description of this modification follows:

The ox pancreas, the kind used in most cases, was obtained at the Chicago Union Stock Yards as soon as the animals were slaughtered, and was cut in small pieces and put in the acid-water-alcohol mixture. Then the pancreas was taken to the laboratory where the liquid was poured off and the pancreas was passed through the meat grinder. The tissue was placed in a refrigerating machine whose temperature was 10 degrees below zero with the hope that the cell membranes of the islet cells might be ruptured by freezing and thawing. The tissue was kept in the frozen state for 12 to 24 hours. Thawing was allowed to take place at room temperature. After this the liquid, which was poured off prior to the freezing and kept at room temperature, was now added to the tissue and increased to meet the requirements of the liquid per each kilogram of pancreas tissue. The Doisy, Somgyri and Shaffer method was then followed rather closely until we came toward the end of the process. By this method we have to date prepared twelve lots of insulin from twelve separate lots of fresh pancreas. The separation of the toxic and the antidiabetic fractions, and the physiological assay of these fractions were made on each lot separately.

Toxic fraction. In using the above method of preparing insulin it was noticed that there were two distinct precipitates thrown down in the final precipitation when nine volumes of alcohol were added to the liquid which had been centrifuged. Naturally, one would want to know the nature of each precipitate and to see if they could be separated. Fractional precipitation suggested itself first. One volume of alcohol was added and a dark gray colored precipitate was thrown down. This was filtered and the precipitate was found to be readily soluble in water. Then eight volumes of alcohol were added to the filtrate. To our surprise only one precipitate was now thrown down and it was snowy white as contrasted with the grayish color given by the two precipitates thrown down together when the nine volumes of alcohol were added in the above operation. The second precipitate was also very readily soluble in water.

Then the nature of the two precipitates was investigated. A biuret test was made on the first precipitate and found to be positive. When the test was applied to the second precipitate only a very slight tinge of pink was noticed. Then a biuret was applied to some of the Lilly Company's iletin lot no. 67872-725710 which had just been received. It was positive, falling somewhere between the first and the second precipitates.

The first precipitate was then tried on rats and guinea pigs. Sufficient water was added to dissolve the precipitate. The first precipitate obtained 1 kgm. of pancreas required about 30 to 50 cc. of water to dissolve it. Two cubic centimeters of this resulting liquid were injected into the peritoneal cavity of an adult albino rat. Two minutes later muscular incoordination was manifested. The rat lay on its side prostrated and breathed heavily. After a few gasps it went into terrific spasms and was dead within eight minutes after the injection. The dose was reduced to $\frac{3}{4}$ cc. and the effects were indentical. This experiment was repeated a number of times with the same results in every case. From now on we will refer to this first precipitate as the "toxic fraction."

To eliminate the possibility of these spasms being due to hypoglycemia, which did not seem likely in this short period, 1 to 3 cc. of a 20 per cent dextrose solution were injected before or at the same time that the toxic fraction was administered. This had no effect on the course of the intoxication which always resulted in death in a very short time.

This toxic fraction was then boiled for five minutes but its toxicity was not affected. That is, it produced the same symptoms upon injection into rats and guinea pigs. Some of it was kept at room temperature for two months and some was kept in the ice box for a similar length of time without its toxicity being appreciably decreased in either case.

Experiments were now performed to determine the minimum fatal dose of this toxic fraction. When $\frac{3}{4}$ cc. was injected the rats showed similar

symptoms but they came on more slowly and proved fatal in ½ to 2 hours. They all died with typical spasms and peculiar gasps resembling asphyxia. Sometimes the rats would squeal as if experiencing pain.

A number of normal rats were killed and their blood sugar was determined. Their values generally fell around 0.070 to 0.080 per cent. When the dose of the toxic fraction was sufficient to kill them in seven to ten minutes there was little effect on the blood sugar, sometimes even a small rise (0.090 per cent). Then the dose was considerably increased and often more than doubled. In one case a very large rat was injected with 2 cc. After four minutes voluntary muscular control was lost. It gradually grew weaker, but did not show very marked spasms. It died ½ hour after the injection with a blood sugar of 0.231 per cent. Another average-size rat was injected with 2¼ cc. of the toxic fraction. Death followed in eight minutes. Its blood sugar was 0.131 per cent.

When the toxic fraction was injected into rats the marked dyspnea indicated a stimulating action on the medullary centers. It was then decided to study the effect of this toxic fraction on animals whose respiratory center was artificially depressed. For this purpose rats and guinea pigs were placed under deep ether anesthesia. One rat was put under ether and injected with 2¼ cc. of the toxic fraction intraperitoneally. The anesthesia was continued for twenty minutes and then the ether was removed. The recovery was slow because deep anesthesia had been maintained, but no signs of spasms were manifested. Another rat was injected with 2¼ cc. This one was not given ether until the spasms became manifest. Ether was administered promptly. As anesthesia was produced, the spasms were allayed and the breathing changed from gasping, jerky movements to a regular even respiration. A third rat was given ether when the spasms were beginning to appear, with similar results. The ether was promptly removed, the spasms soon returned and the gasping respiratory movements appeared again. The animal was allowed to hover between these two stages for twenty minutes which shows that the fatal symptoms were due to excessive stimulation of the medullary centers. All the rats appeared depressed for several hours afterward, but gradually returned to normal. The above experiments were repeated on a number of rats with similar results. Following this 2¼ cc. of the toxic fraction were injected into each of three unanesthetized adult rats and each succumbed in less than eight minutes with typical spasms.

Guinea pigs were used with similar results. They, too, suffered muscular incoördination within three to five minutes after the injection. Very often they would die within eight minutes after the toxic fraction was injected, in spasms as described for the rats. As the dose was decreased the animals lived proportionately longer, but died in typical spasms sometimes twelve hours after the injection.

The experiments with the toxic fraction were extended to dogs and rabbits. A 3-kgm. rabbit was allowed to inhale a very little ether, just sufficient to produce analgesia, while the carotid artery was cannulated for recording the blood pressure. The ether was taken away and the toxic fraction injected intraperitoneally and its effect noticed on the blood pressure tracing. There was a continuous gradual fall of the blood pressure until the end. The blood sugar, as determined from a sample of blood taken from the heart immediately after death, was 0.324 per cent. This high blood sugar cannot be accounted for entirely by the effects of ether because the amount given was very small. This operation was repeated and the animals behaved in a similar fashion. The blood sugar was 0.312 per cent. Another rabbit was injected with 4 cc. of the toxic fraction subcutaneously. It died two hours later but not in spasms as did the rats and guinea pigs. The blood sugar from the heart was 0.217 per cent. Another rabbit died in fifty minutes with a blood sugar of 0.373 per cent as compared with its normal of 0.135 per cent.

It might be mentioned in this connection that a number of rabbits were killed by the product obtained when an attempt was made to manufacture insulin by the Murlin method. In one case a rabbit was injected intraperitoneally with 4 cc. of the final product. Death followed in thirty minutes. The blood sugar was 0.357 per cent. In a similar case a large rabbit received 10 cc. intraperitoneally. Three hours later the blood sugar was 0.735. An hour later the sugar fell to 0.277 and the rabbit died shortly thereafter. Later a means was discovered by which the toxic fraction could be removed by a modification of the Murlin method.

From the above experiments it is evident that there are two antagonistic substances in the pancreas extract, the one tending to lower the blood sugar, the other producing a rise in blood sugar. The toxicity of the substance which produced the rise in blood sugar was clearly demonstrated for rats, guinea pigs and rabbits. Now the question arose whether this toxic fraction might be responsible for the local irritation, and the sterile abscesses and the indurations at the sites of injection as reported in many cases. The iletin obtained from the Lilly Company in January, lot no. 67872-725710, was used but a few days to treat a diabetic dog by subcutaneous injection when abscesses appeared at the site of injection. In six other diabetic dogs treated with insulin, preserved with tricresol from which the toxic fraction was removed, there was never any induration nor a sign of an abscess. Then the toxic fraction was injected subcutaneously into a dog with pancreatic diabetes of four weeks standing. In each case 2 cc. were injected. The blood sugar was not affected, but two days after each of three injections at different places, there appeared an abscess at the site of each injection. The area was always sterilized and likewise the needle before the injection was made. One other diabetic dog which had been

treated with insulin for four weeks without the appearance of any local inflammatory reaction, was given three injections of a product from which the toxic fraction was not all removed. Two days after each injection an abscess appeared and then opened leaving an ulcer about one inch in diameter. To this dog insulin was then given which had been freed from the toxic fraction without any further local reactions being produced. A normal dog was then given subcutaneous injections of the toxic fraction on one side of his back and at the same time injected similarly with insulin from which the toxic fraction was removed, on the other side. No reaction appeared where the insulin was injected. At the site of the injection of the toxic fraction there appeared a hard knotty lump. In every case aseptic precautions were taken and the substances injected were almost neutral. The effects produced were not due to trieresol as this was carefully controlled by not adding the preservative to the toxic fraction. One diabetic dog has already received two injections per day of insulin freed from the toxic fraction for two hundred and ten days without the appearance of any local reaction. All of the injections were made in two areas each about three inches in diameter on each side of his vertebral column behind the scapula. The cases cited above indicate that the toxic fraction is responsible for the local reaction produced by commercial insulin. However, I am not ready to make that assertion although the cases thus far tried point that way. There is a small amount of insulin removed with the toxic fraction and work is under way to reduce this to a minimum.

The active fraction. The precipitate thrown down by the addition of eight volumes of 95 per cent alcohol, after the precipitate formed by the addition of one volume of alcohol was removed, is a flocculent, snowy white precipitate and shall be designated as the active fraction. It, too, is very readily soluble in water. This second precipitate from a kilogram of fresh pancreas was of such a bulk that 70 cc. of distilled water were used to dissolve it.

To standardize the insulin it was thought that it would be well to compare it with the Lilly product which had just been received. A rabbit weighing 2.4 kgm. was taken from its cage, in which food is kept constantly, and his normal blood sugar determined. Samples of blood were taken at definite intervals in order to follow the action of the Lilly iletin. The rabbit was not fed during the experiment. In the evening after the experiment it was returned to the cage at which time it ate greedily. In the morning the same rabbit was treated similarly with the exception that it was injected with 1 cc. of the active fraction just prepared.

One cubic centimeter of the Lilly product lowered the blood sugar from 0.172 to 0.080 in five hours. One cubic centimeter of the active fraction lowered the blood sugar from 0.200 to 0.073 in five hours. Judging from this the 1 cc. of the active fraction was slightly more potent than 1 cc.

of the Lilly product which contained ten units per cubic centimeter. To be conservative we will consider that 1 cc. of this active fraction contains 10 insulin units. Then the 70 cc. made from 1 kgm. of pancreas would contain 700 units. This was an early sample of iletin manufactured by the Lilly Company, with which we compared our product, so no conclusions were drawn as to its present potency, since the early iletin samples were of uncertain potency. Later we standardized our active product against samples of iletin tried on human diabetics, and on totally depancreatized dogs according to the method of assay of Wilder. Normal rabbits were also used and the three methods checked rather closely according to biological methods of assay. The results of these three methods of assay furnished a means whereby the actual potency of the active fraction could be calculated.

In every case where ordinary ox pancreas was used the number of units obtained from a kilogram of pancreas usually varied between 650 and 800 units. This variation may be accounted for in part by varying amount of fat that was left on the pancreas. On the whole, the amount obtained per kilogram is comparatively constant. There were two wide variations from these results which might be mentioned.

On one occasion when I went to the stock yards for material, only very old cows were being slaughtered. This afforded an opportunity to learn the insulin content of the pancreases of old animals. Ten pounds were obtained and taken through the process in three lots. Very little insulin was obtained from the ten pounds—probably 100 units per kilogram. In the other case, the variation was in the opposite direction. Calves' pancreas had been obtained and treated by the same method. The yield in this case was 1000 units per kilogram. These unusual cases will be repeated at an early date.

In light of the consistency of the results obtained with beef pancreas one is justified in concluding that the greater potency obtained per kilogram of pancreas is in large part accounted for by removing the toxic fraction which raises the blood sugar. When it is not removed the insulin is proportionately reduced in potency according to the amount of the toxic fraction present, because of its power of raising the blood sugar.

Extracts of liver, kidney, testis and adrenal were treated by the above method. There was a toxic fraction present in each organ which was somewhat similar to that obtained from the pancreas. A final precipitate was obtained from each organ which was almost identical with the corresponding precipitate (insulin) as to appearance and amount. This final precipitate in the case of the organs other than the pancreas had some effect on the blood sugar of rabbits. This phase of the work is being made a special study of in this laboratory by Mr. J. S. Ashby.

DISCUSSION. It was in following the procedure furnished me by Doctor Shaffer that my attention was attracted by the two distinct precipitates thrown down by the addition of nine volumes of 95 per cent alcohol in the final precipitation. Fractional precipitation was tried and proved successful in separating the toxic fraction from the active fraction. The toxic fraction was shown to raise the blood sugar in guinea pigs, rats and rabbits as well to possess a marked stimulating action on the medullary centers. The latter fact was demonstrated by showing that its stimulating action did not result in death when the medulla was markedly depressed by ether anesthesia. That the toxic fraction is responsible for the production of local irritation and sterile abscesses, as described by various clinicians, is strongly indicated. The preservative tricresol was considered by some to be the cause of the sterile abscesses, but the toxic fraction will produce the abscesses when no preservative is used.

As a result of isolating and removing this toxic fraction one is able to secure a greater yield of insulin per kilogram of pancreas than has been possible heretofore. This is what one would expect since these two substances are antagonistic—the toxic fraction producing a rise in the blood sugar and the active fraction causing a lowering of blood sugar. The active fraction has never given evidence of the production of local irritation or of abscesses. This has been tried on eight diabetic dogs. The one dog received two injections per day for two hundred days. All the injections were made in one area about three inches in diameter, with the idea of fairly testing the irritating effects of the active fraction, if it possessed such properties.

The yield of 1000 units or more per kilogram of calf pancreas and the yield of about 100 units per kilogram of old cow pancreas needs to be confirmed. The consistency of obtaining 650 to 800 units from each kilogram of ox pancreas would seem to favor the correctness of the results obtained in the above two cases.

CONCLUSIONS

1. There are present in the pancreas two antagonistic substances. The one substance, called the toxic fraction, raises the blood sugar and causes death in rats and guinea pigs, by the excessive stimulation of the medullary centers. The other material, called the active fraction (insulin), is the specific antidiabetic substance.

2. The yield of insulin per kilogram of pancreas is proportional to the degree to which the toxic fraction is removed.

3. The toxic fraction seems to be responsible for the irritation and sterile abscesses produced at the sites of injection. The active fraction causes no such changes.

4. One lot of pancreas obtained from old cows yielded very little insulin (100 units); another lot obtained from calves yielded 1000 units per kilogram.

5. The greater part of the final alcohol precipitate, even after removal of the toxic fraction, appears to be something else than insulin, since mammalian organs (kidney, liver, suprarenal, gastric mucosa and testis) other than the pancreas handled by the above method yield water soluble precipitates of similar appearance and quantity, but showing much less insulin action.

I wish to express my thanks to Dr. A. J. Carlson for his helpful criticisms during the progress of this work and for his revision of the manuscript.

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II. THE ABSORPTION OF INSULIN FROM THE INTESTINE, VAGINA AND SCROTAL SAC

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Following the discovery of insulin and its value established in the treatment of diabetes mellitus, many methods of administration were tried. The methods of inunction, per rectum, intraperitoneally, intravenously and orally were not found satisfactory. Murlin and Gibbs (2) found a slight lowering of the blood sugar when comparatively large amounts of insulin were given by duodenal tube. There was also an increase in the respiratory quotient. When the insulin was given by mouth and in salol-coated pills no effect was noticed. Joslin (3) reports negative results from two diabetic patients to whom insulin was given by mouth. In a very recent communication Mills (6) reports that he could prevent the usual rise in blood sugar due to ether anesthesia by introducing insulin into the intestines of rabbits. He also found that insulin given by mouth to diabetic patients and to normal individuals had no effect on blood sugar. Woodyatt (8) reports negative results when insulin was administered by the oral, intranasal, rectal and vaginal routes. Inunction was also tried with similar results.

In view of the above facts, Doctor Carlson suggested that insulin might be effectively administered by a fistula of the appendix, since it affords a splendid place for absorption and is probably comparatively free from destructive enzymes. It was with this in mind that the work was undertaken.

The rabbit was thought to be a suitable animal but proved unsatisfactory for testing the effects of insulin when administered by the fistula of the appendix. As the rabbit is hyper-excitable the desired relaxation could not be obtained and when insulin was introduced into the fistula there was violent contracture of the abdominal musculature with consequent expulsion of the insulin.

A Thiry fistula in the ileum of a dog then suggested itself as a possible means of solving this problem of intestinal administration. Several Thiry fistulae were made and the dogs allowed to recover. This usually required five to ten days. Total pancreatectomy was then performed and about four days later insulin was administered by fistula. The degree of lowering of the blood sugar was taken as a measure of the effectiveness of the insulin.

METHOD OF ADMINISTRATION. The dog was placed on his left side on very thick pads of cotton on a trough-like table. In this position the fistula was uppermost since it was brought to the outside, not through the operative wound, but by way of a small stab wound high on the right side. Thus little opportunity was afforded for the insulin to escape, especially since the segment of intestine was reversed so that the direction of the peristaltic movements was inward. That the animals were in the best possible condition for aborption to take place can be judged from the fact that the animals always lay very quiet and often fell asleep during their four to six hours' stay on the table.

Before giving insulin the segment of the intestine was washed our with various solutions to free it from the yellow cheesy substance which was constantly secreted by the intestinal mucosa. A stiff rubber tube about 12 inches long with a very small lumen was coated with vaseline and inserted to the bottom of the fistula. To this tube a 10 cc. syringe was attached and the insulin introduced a few drops at a time, while constant watch was kept on the opening to note any escape of the insulin, should this happen. Many times such a degree of muscular relaxation was manifested that not a drop of liquid would escape from the fistula in six hours. The blood samples were taken either from the front or hind leg vein without eliciting the slightest resistance on the part of the dog.

Table 1 gives the blood sugar changes after the administration of insulin by Thiry fistula. From it the reader will note that the blood sugar was lowered rather promptly by insulin introduced into the intestinal fistula but remained at the new level for only a short while. This temporary effect on the blood sugar suggested that other factors besides insulin were operating. Possibly the alkaline juices of the gut destroyed the insulin before it was absorbed and so only a small portion of the injected material ever entered the blood stream. There is also a possibility, and there is some evidence for this, that the insulin was absorbed very rapidly and excreted by the kidney before it had sufficient time to produce a lasting effect.

To see whether the alkalinity of the intestinal juices was a factor, the gut was washed with 0.1 or 0.2 per cent HCl before introducing the insulin. Sometimes this procedure yielded very gratifying results, but not in every case. On the other hand, according to Lamb and Smith, acid in the intestine in certain concentrations, produces inflammation and would naturally modify the absorptive power of the mucosa. In our experience, we obtained better results when the gut was irrigated with 0.1 per cent than when 0.2 per cent HCl was used. In the latter case the mucosa appeared cyanotic and the dog became very restless which had not been observed when weaker acids were used.

Administration of insulin per vaginum. In the light of the results obtained with a Thiry fistula, the possibility of administration of insulin per vaginum suggested itself. A young bitch weighing 9 kgm. was prepared by a simple operation that is ordinarily performed to facilitate catheterization. This was necessary to make certain that the insulin was introduced into the vagina and not into the bladder. The wound healed very rapidly. Ten days later the dog was depancreatized. The excretion of sugar was controlled by subcutaneous injections of insulin

TABLE 1

Total pancreatectomized dogs

Details of typical experiments. Each of the experiments listed below was repeated at least four times, with similar results.

1. 350 rabbit units of insulin by Thiry fistula in 2½ hours. The toxic fraction had not been removed in this one case

Blood sugar	0.431	0.266	0.139	0.033	0.208	0.217
Time	0	1¼ hrs.	2¾ hrs.	4¼ hrs.	5½ hrs.	7¾ hrs.

2. 30 rabbit units (3 cc.) of insulin introduced into the Thiry fistula at one time

Blood sugar	0.641	0.510	0.485	0.300	0.563
Time	0	1 hr.	2 hrs.	3½ hrs.	4¾ hrs.

3. 300 rabbit units (30 cc.) diluted to 70 cc. and very slowly introduced by Thiry fistula, the total time being 2 hours

Blood sugar	0.241	0.198	0.220	0.105	0.111	0.333
Time	0	2½ hrs.	3 hrs.	4½ hrs.	6 hrs.	7¼ hrs.

4. 77 cc. crude extract, which would yield about 100 units, introduced very slowly by Thiry fistula in 2 hours

Blood sugar	0.307	0.289	0.173
Time	0	2¾ hrs.	5 hrs.

until the abdominal wound was healed, which required almost one week. The insulin used for subcutaneous injections was the same kind as that used in the vaginal administrations. For this reason we secured a comparison of the effects produced by insulin when introduced by different routes. By injecting 1.5 cc. of insulin into this dog subcutaneously twice a day, her urine was kept sugar-free. The effects produced by the varying amount, when given by way of the vagina can be seen from table 2.

From table 2 one can see that the maximum effect is produced about three hours after the administration of insulin and the blood sugar returns to normal rather rapidly, resembling the effect produced when it was given

by the Thiry fistula. When 12 cc. of insulin were introduced in 4 cc. doses at 1 to 1.5 hour intervals the blood sugar was kept at the low level for a proportionately longer period of time. The lowest point was reached about 3.5 hours after the last administration. Then the blood sugar rapidly rose and in one hour and forty-five minutes reached the diabetic level of more 0.600.

By this method of administration the reduction in blood sugar takes place earlier than by subcutaneous injection, but it is of shorter duration. It was this repetition of the temporary lowering of blood sugar, as seen in the case of the Thiry fistula, that forced me to look for the excretion of insulin in the urine, since it appeared that the hormone was very readily taken into the blood stream, produced its effect and was rapidly excreted. That this line of reasoning was correct is shown by the fact that insulin can easily be recovered from the urine of such animals as well as from that

TABLE 2

Total pancreatectomized dogs

Details of typical experiments. The experiments were repeated several times with similar results.

1. 20 rabbit units (2 cc.) introduced into the vagina at one time

Blood sugar	0.622	0.622	0.106	0.416	0.463
Time	0	1¾ hrs.	3 hrs.	4¼ hrs.	5½ hrs.

2. 120 rabbits units (12 cc.) introduced into the vagina in 2½ hours

Blood sugar	0.650		0.143	0.103	0.675
Time	0	2¾ hrs.	4 hrs.	6 hrs.	7¾ hrs.

of human diabetics treated by insulin, but a full report of this will appear in a later communication.

Moreover, I am in a position to state that when insulin is injected into the blood stream it is recovered almost quantitatively from the urine. It produces its effect and apparently is not changed during the time it remains in the circulation. When recovered from the urine and re-injected into rabbits, insulin produced hypoglycemic convulsions which are readily relieved by the injection of dextrose. Some of it can be recovered from the urine of the dogs which have been treated with insulin by Thiry fistula. Less is recovered from the urine of dogs which have received subcutaneous injections of insulin.

These facts seem to indicate that when insulin is placed in contact with a mucous surface it gains entrance into the blood stream very readily and is excreted in the urine in large amounts. When it is injected subcutaneously it is absorbed more slowly and probably some of it is destroyed in the subcutaneous tissues themselves before it has a chance to enter

the blood stream and this may make the actual dose much smaller than that injected. This would explain the presence of insulin in the urine in such small amounts following subcutaneous injections. The evidence favoring this is, that when insulin is injected into the blood stream it is apparently unchanged and is excreted in the urine almost quantitatively. Now, if insulin is changed but little in the blood stream, and we cannot recover half the amount from the urine that has been injected subcutaneously, we are justified in concluding that insulin is changed in some way or destroyed in part before it reaches the blood stream.

On the other hand it is quite possible that during the normal continuous activity of the islet cells of the pancreas a certain definite amount of insulin is delivered into the blood per unit times and circulates in it in a certain concentration before it is absorbed by the various organs of the body. The continuous production and utilization of insulin tends to keep its concentration in the blood nearly constant and whatever that value may be we may call it the threshold value insofar as the kidney is concerned. Now, flooding the blood with insulin when it is given intravenously or by a route that permits a very rapid absorption into the blood stream—vagina, intestinal fistula—results in this threshold being exceeded many times, and while it undoubtedly has a rapid and decisive effect, this effect is short lasting because the kidney excretes the excess insulin. However, when it is given subcutaneously and the absorption is very slow it may be entering the blood stream at about the same rate at which it is normally delivered by the pancreas in healthy animals, may be taken up by the tissues and never exceed this threshold value, or else exceed it to a small extent. This would readily explain why it could be recovered from the urine in the former cases and not in the latter. In reality, both of these factors may be operating in bringing about the condition described above.

Administration by scrotum. In looking for a method to parallel the vaginal administration in the female, the scrotal sac suggested itself as a feasible one. The vascularity of the scrotal area would seem to be conducive to rapid absorption and this proved to be true.

Method. The rabbit appeared to be the ideal animal for testing this since it possesses a patent inguinal canal. The testis was gently pushed up into the abdomen and insulin injected into the scrotal sac. This procedure furnished very good results as will be seen from table 3.

From the results in table 3 we see there are several points in favor of administration by scrotum. In the first place it acts more promptly, as one would expect, because of the increased vascularity, as compared with the subcutaneous tissue. Another thing in favor of this method is that the animal does not seem to experience any irritation as is often the case after subcutaneous injections. In addition to the above points it acts for a comparatively long period of time. This was especially marked in one

case where a 2-kgm. rabbit which had not been starved, was injected with 1.5 cc. of insulin. Within two hours the rabbit was in convulsions and continued going into convulsions following administrations of dextrose after a period of 12 hours. Eleven grams of dextrose were not sufficient to neutralize the effects of insulin which had continued acting even after twelve hours. The fact that it is absorbed slowly is indicated by the duration of the action. If it were absorbed rapidly its effect would be very marked but of short duration as in the case of the Thiry fistula. It appears that although it is rapidly absorbed at first the rate of absorption is considerably diminished later on. That is the only way we can explain its rapid action and long duration of the hypoglycemia.

TABLE 3

Normal rabbits

1. Injected 1.5 cc. of insulin (about 22 rabbit units, as compared with Lilly's iletin) into the scrotum of a 3.5 kgm. rabbit which had not been starved

Blood sugar	0.132	0.054	0.042*
Time	0	¾ hr.	2 hrs.

2. Injected 6 rabbit units (⅔ cc.) of insulin into the scrotum of a 2.5 kgm. rabbit which had been starved for 24 hours

Blood sugar	0.120	0.055	0.041†	0.027	0.044
Time	0	3 hrs.	4½ hrs.	7 hrs.	8½ hrs.

* Rabbit went into hypoglycemic convulsions at one-hour intervals as long as observed (12 hours). Each time the convulsions were relieved by injecting intravenously 1 gram of dextrose. Twelve grams not sufficient to protect; rabbit died during the night.

† Rabbit went into mild convulsions at short intervals but recovered each time without injecting dextrose. Fifteen units of Lilly's iletin were required to produce a similar effect on this rabbit.

Discussion. Of the three methods of administration the scrotal method seems to be the best. By this means the action is prompt, long continued, and the maximum good derived from a certain amount of insulin. This method was tried on rabbits only and no attempt has been made to see what quantity could be recovered from the urine. To be sure we are not recommending this method at once for the treatment of human diabetics. However, in cases requiring constant administration of insulin the scrotal method may be even better than the subcutaneous method as far as the amount of irritation produced and the maximum benefit derived from a unit of insulin are concerned but affording a better chance of infection because of its greater vascularity.

The results obtained with a Thiry fistula are indicative of what might be expected from administration by means of the fistula of the appendix.

Insulin is readily absorbed from the intestine, lowers the blood sugar and is rapidly excreted by the kidneys. Doubtless more gratifying results will be obtained from the Thiry fistula method when the rate of absorption is controlled and the action of the destructive enzymes inhibited.

A factor in favor of administration by the Thiry fistula is that crude extracts can be used with fair success and no concern need be exercised about having the toxic fraction or the proteins removed.

The vaginal method yields results similar to the Thiry fistula method in regard to quick absorption and a temporary action. However, the effect may be prolonged by administration at intervals of one and one-half hours. This method is too dangerous to be of clinical significance, hence it is not recommended.

SUMMARY

1. Injections of insulin into the scrotal sac yield the maximum effect per unit of insulin, a prompt action, and of long duration, without apparent irritation.

2. The very temporary action of insulin when introduced into the gut seems to be due in part to rapid elimination by the kidneys, suggesting too rapid, rather than too slow absorption.

3. Insulin given by the vagina is absorbed and produces a temporary lowering of the blood sugar. The low level may be maintained by introducing insulin at one and one-half hour intervals.

I wish to acknowledge my indebtedness to Dr. A. J. Carlson for suggesting this problem and for his continuous interest and helpful criticism during the work. Thanks are due Mr. Fred T. Purdum and Oscar Almquist for the able assistance they rendered me in the course of this research.

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III. EXCRETION OF INSULIN BY THE KIDNEYS

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The temporary effect produced on the blood sugar by insulin, when given by Thiry fistula, led Doctor Carlson to suggest that insulin might be rapidly excreted by the kidneys. The administration of insulin per vaginum in dogs also produced a similar effect on the blood sugar. This repetition of the previous effects warranted our attempt to look for the insulin in the urine.

Method. As a step toward the isolation of insulin from the urine, a known amount of insulin was added to a certain volume of normal urine and recovery of the insulin was attempted. At this time we were using the Shaffer method of preparing insulin, so we naturally followed this procedure in recovering insulin from the urine. By half-saturating the urine-insulin mixture with ammonium sulphate we hoped to separate the insulin with the proteins to which it always seems to be adsorbed. From this point on the procedure was successful as judged by the effects of injecting the recovered insulin into rabbits. As nearly as can be determined by the rabbit assay, all the insulin added to the urine was recovered. This was repeated several times with similar results. With a method yielding such satisfactory results, we began to apply it to the urine of patients and animals to whom insulin had been administered by various routes.

1. Insulin was injected intravenously in a dog under veronal to study its effect on respiration, blood pressure and blood sugar. The normal blood sugar was determined and hourly samples of blood drawn thereafter. One cubic centimeter (ten rabbit units) of insulin was injected at one hour intervals until the dog died. The blood sugar fell gradually, as was expected, until a per cent of 0.012 was reached, at which point the respiration stopped while the heart beat feebly for a short while.

Four injections of insulin were made, and the animal died about forty-five minutes after the last injection. If the insulin is excreted by the kidney, we would not expect all of the last injection to be excreted in this short time. The urine was taken from the bladder of this dog and, to our surprise, we obtained from it 35 units of insulin (40 units altogether injected). This experiment was repeated on a dog of the same size with

identical results. From these two experiments we see that in normal dogs, under veronal, insulin produces its effect on the blood sugar, and is rapidly excreted. It produces its usual effect on the blood sugar of the dog and, when recovered from the urine, it will produce as great an effect on the blood sugar of rabbits as the same amount of insulin before it was injected into the dog.

2. The urine of a patient with marked diabetes mellitus, not receiving insulin treatment, was examined to see if the kidney of diabetics possessed a greater permeability than normal for the insulin, which might still be furnished by the pancreas. No trace of insulin was obtained from the urine of this patient. The patient was given 3.8 cc. (38 units) of Lilly's iletin in two subcutaneous injections during one day. The urine was collected for the thirty-six hours following the first injection. From this urine we recovered 6 units, or about one-sixth of the amount injected. Similar results were obtained with three diabetic dogs when insulin was administered by the subcutaneous route.

3. From the urine of dogs given insulin by the vaginal or Thiry fistula route we were able to recover more insulin than when the insulin was administered subcutaneously. This seems to indicate that the rate of elimination of insulin by the kidneys depends upon the rate at which it enters the blood stream. Probably there is a normal insulin threshold for the kidney and when this is exceeded, as when insulin is injected directly into the blood stream, or is rapidly absorbed from the vagina or Thiry fistula, it is rapidly excreted and its action is only temporary. When insulin is injected subcutaneously it is absorbed into the circulation probably at a rate closely corresponding to the normal condition and does not exceed the insulin threshold, with the result that little is excreted in the urine. This would explain the finding of insulin in amounts varying with the rate at which it enters the blood stream.

When these experiments were completed we read in a preliminary communication by Best and Scott[1] that they had found insulin in the urine of diabetic patients only after being treated with insulin. In this respect their findings agree very well with ours. They also report an insulin-like substance in normal urine. We have not detected such a substance in the urine of three normal individuals.

4. When we found that the amount of insulin present in the urine seemed to vary with the rate at which it entered the blood stream we were anxious to know how much insulin could be recovered from the urine when insulin was administered orally. To test this we selected a 11-kgm. dog and starved him for twenty-four hours. At the end of the twenty-four hours his normal blood sugar was determined and he was then given 70

[1] Best and Scott: Journ. Amer. Med. Assoc., 1923, lxxxi, 382.

cc. (700 rabbit units) of insulin by stomach tube. The only symptoms manifested were intermittent periods of shivering which lasted but a short time. Four hours after the administration of the insulin the blood sugar was 0.166 as compared with the normal of 0.090. A recent paper by Mills reports similar findings in normal and diabetic individuals. No explanation is offered by Mills for this rise in the blood sugar.

The dog to which the insulin was given by mouth did not micturate until twenty hours later. From this urine we recovered one-fourth the insulin administered. About the same proportion of the insulin was recovered from the urine of other dogs when insulin was given in large amounts by mouth, as seen in table 1.

A dog about four months old, weighing 3 kgm., was selected to determine whether it was possible to produce hypoglycemia by the oral administration of insulin in young animals. The greater permeability of the intestinal mucosa of the young animal was thought to be favorable

TABLE 1

Normal dogs. Large quantities of insulin by stomach tube

WEIGHT OF DOG	BLOOD SUGAR		INSULIN BY MOUTH (RABBIT UNITS)	INSULIN IN URINE (RABBIT UNITS)
	Normal	1 hour after insulin per os		
kgm.				
10	0.100	0.110	800	150
12.5	0.096	0.102	1000	200
9	0.090	0.100	700	150

to this end. The pup was starved for twenty-four hours and his normal blood sugar determined and found to be 0.111. Then 700 units of insulin were given by mouth. The dog vomited forty-five minutes later, could not stand up, and manifested the typical hypoglycemic symptoms. He was watched very carefully, but no dextrose was administered. Fifteen minutes after that the dog showed signs of recovery; the blood sugar was 0.039. This demonstrated that it was possible to produce hypoglycemic conditions when insulin is administered orally in large quantities to young dogs. About one-fourth of the insulin given by mouth was recovered from the urine of this dog.

Next, we wanted to know how much insulin was required to produce an effect on the blood sugar of this dog without producing convulsions. Also we desired to compare the effectiveness of insulin in the young with a similar amount in the adult. We administered to the young dog by stomach tube 175 rabbit units of insulin, or the same amount per kilogram body weight that was given to the adult dog, weighing 11 kgm., in the previous experiment. The pup was starved twenty-four hours, as in the previous experiments. The blood sugar was found to be 0.099

before the administration of the insulin. One hour later the blood sugar level of 0.060 was reached.

Then an adult dog was selected weighing 3.6 kgm. It was prepared similarly to the other dogs for the oral administration of insulin. We gave this dog 900 units of insulin by stomach tube, or 200 units more than we gave the young dog weighing 3 kgm. The normal blood sugar was 0.080 and there was no change in the blood sugar one hour after administration. This shows that more insulin is required to produce a lowering of blood sugar in adult dogs, if this effect can be produced in adult dogs when insulin is given by mouth.

Discussion. From these experiments it is evident that an insulin-like substance can be recovered from the urine of diabetic patients and normal and diabetic dogs only after they have been treated with insulin. The amount that can be thus recovered seems to vary with the rate at which the insulin enters the blood stream. When insulin is administered by Thiry fistula or vagina, proportionately more is recovered from the urine than when it is administered subcutaneously. About one-fourth of the insulin can be recovered from the urine of the normal dog when given by mouth. When insulin is injected into the circulation of a normal dog under veronal anesthesia, it is rapidly excreted by the kidneys and almost the entire amount can be recovered from the urine in the bladder. This demonstrates that when insulin is introduced into the blood steam at a rapid rate it produces its usual effect of lowering the blood sugar and is excreted by the kidneys in proportion to the rate at which it enters the blood stream. This seems to indicate that there is a certain insulin threshold possessed by the kidney and when this is exceeded the insulin is eliminated. In support of this idea is the fact that a large amount of insulin can be recovered from the urine of diabetic dogs when it is administered by Thiry fistula or vagina where absorption is rapid and the threshold is soon exceeded.

When insulin is administered orally to normal adult dogs, we are able to recover about one-fourth of it from the urine. It is therefore absorbed, yet does not produce a lowering of the blood sugar while in the blood stream. No explanation is offered for this unless it is a question of existing saturation of the liver and muscles with glycogen. But it seems that a great excess of insulin in blood is required for lowering of the blood sugar in the normal dog. In the case of young dogs a lowering of the blood sugar, even to the point of hypoglycemic convulsions, can be induced by oral administration of insulin. The readiness with which oral administration of insulin lowers the blood sugar in the young dog may be due to the greater permeability of the mucosa of the alimentary tract in the young animal. When the destructive enzymes of the alimentary tract and the rate of absorption can be controlled, oral administration may be practicable in diabetic children.

SUMMARY

1. Insulin can be recovered from the urine of diabetic dogs in amounts varying with the rate at which it enters the blood stream. More insulin can be recovered when it is given by the vaginal, oral, or Thiry fistula route or by mouth than when administered subcutaneously.

2. Large amounts of insulin administered orally will produce hypoglycemic convulsions in normal pups. Proportionately larger amounts will produce a rise in the blood sugar of normal adult dogs, similar to Mills' findings in normal and diabetic individuals.

IV. AN INSULIN-LIKE SUBSTANCE IN THE KIDNEY, SPLEEN AND SKELETAL MUSCLE

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The important discovery of Banting and Best immediately raised the question whether insulin or any other sugar-reducing substance could be isolated from various other organs of the body, aside from the pancreas. Should such sugar-reducing principles be found in other tissues, it would be important to determine whether it is the insulin formed by the pancreas and merely stored there, or whether the cells of the organs themselves are capable of producing an enzyme or substance that would enable living cells to oxidize, or polymerize glucose. Unicellular and multicellular lower animals have no pancreas and are undoubtedly able to utilize glucose for energy purposes. But the tissue of higher animals, with the advent of the islands of Langerhans, may gradually lose this property.

The discoverers of insulin performed some experiments (1) to elucidate this point. They employed extracts of liver, thymus, kidney and thyroid of cattle and found that none of these extracts, with the exception of the thyroid, where very slight results were obtained, lowered the blood sugar of a diabetic dog. They conclude, therefore, that insulin is a specific hormone elaborated by the islet cells of the pancreas. Similar results were later reported by Macleod on fish. Since the conclusion of our work a preliminary report by the above investigator states the finding of an "insulin-like substance" from nearly all animal tissues. That living cells in general do contain an insulin-like principle was first shown by Collip (3), who extracted a sugar reducing principle from yeast cells, higher plants, and also from the tissues of the clam (2). In the present investigation we prepared extracts from the kidney, spleen and muscle of normal cattle and dogs; we also extracted similar organs of completely depancreatized dogs killed five or six days after the operation. This latter was done in order to determine whether the insulin found in these organs is the hormone produced in the pancreas and stored in the various tissues of the body.

METHODS. Our first attempt to isolate insulin or a similarly acting substance was by the original method of Collip (4) using the kidney and

spleen of normal dogs. We next tried the Murlin method of insulin extraction (private communication to Doctor Carlson) on similar organs and tested our preparation on rabbits, both starved and fed. All injections were made subcutaneously, and the blood for the blood sugar determination was drawn from the marginal ear vein. At this time Shaffer published his method of preparing insulin and on adopting his procedure (5) we obtained our first positive results. However, the results were not satisfactory for there was a preliminary rise in the blood sugar and the ensuing fall was not very marked. Later Fisher, in this laboratory, modified the Shaffer method (6) and it was by the use of this modified procedure that our first real success was achieved. To make our results comparable to the procedure used in extracting insulin, the final precipitate from 1 kgm. of tissue was taken up in 50 cc. of distilled water. Our product was injected into both starved and fed rabbits and their blood sugars were determined by the Folin-Wu method of blood sugar analysis.

Results. Our results indicate that there is insulin or some other closely related substance in the kidneys, spleens and muscles of normal cattle and dog (tables 2, 3).

By the Collip method, as seen in table 1, there was a marked rise in blood sugar at the end of the second hour following injection of 4 cc. of an extract of both dog's kidney and spleen. In the case of the kidney there was a slight fall in the next hour but as this was in the third decimal place it was of no great significance. The spleen extract here acted similarly to the kidney with the exception that at no time did the blood sugar fall below normal. Practically the same result was obtained with the Murlin method of preparation, with the exception that the return of the blood sugar value to normal was still longer delayed, and in no case did it fall below normal (table 1). In the one experiment using Shaffer's method, there was the above noted rise in the blood sugar value at the end of the first hour (table 1) but at the end of the second hour there was a fall from 0.133 gram. This fell to a lower level at the third hour (0.103 gram), but began to rise again at the fourth hour.

In all of the above experiments there was a decided rise in the blood sugar values during the first hour and owing to this fact we concluded that there was a substance that raises the blood sugar content prior to the true insulin effect noted during the second and third hours. Fisher then developed the fractional precipitation method, using equal volumes of 95 per cent alcohol and extract, in the last step of the Shaffer method, and by this means separating out the toxic substance as a dark flocculent precipitate and after filtering this material off, precipitating the purer insulin ("active fraction") by means of 8 to 10 volumes of 95 per cent alcohol. He tried this on pancreatic extracts with very good results (6),

the toxic fraction killing rats in 6 to 7 minutes when given intraperitoneally, and the insulin producing no rise in blood sugar or any other acute toxic symptoms. We then tried this method of preparation on

TABLE 1

Blood-sugar values of rabbits after hypodermic injection of the following extracts

EXTRACT	NORM.	2D HOUR	3D HOUR	5TH HOUR
Injected 4 cc. extract of kidney (Collip)	0.125	0.158	0.120	0.122
Injected 4 cc. extract of spleen (Collip)	0.121	0.143	0.125	0.122
Injected 3 cc. extract of kidney (Murlin)	0.109	0.205		0.139
*Injected 3 cc. extract of spleen (Murlin)	0.138	0.205		0.152
*Injected 2 cc. extract of spleen (Shaffer)	0.133	0.111	0.103	0.124

* Fed rabbits; all others were starved 24 hours.

TABLE 2

Blood-sugar values of rabbits after hypodermic injection of the following extracts

EXTRACT	NORM.	2D HOUR	4TH HOUR	
Injected 2 cc. extract of spleen (Fisher)	0.116	0.073	0.076	
Injected 8 cc. extract of spleen (Fisher)	0.121	0.062	0.065	
*Injected 2 cc. extract of kidney (Fisher)	0.150	0.146	0.138	0.088—9th hour
Injected 2 cc. extract of kidney (Fisher)	0.125	0.086	0.083	0.095—20th hour
Injected 8 cc. extract of kidney (Fisher)	0.123	0.065	0.062	0.077—24th hour
Injected 2 cc. extract of normal muscle (Fisher)	0.116	0.049	0.052	0.112—24th hour
Injected 10 cc. extract of normal muscle (Fisher)	0.121	0.049	0.051	

* Fed rabbit; all others were starved 24 hours.

TABLE 3

Blood-sugar values of rabbits after hypodermic injection of the following extracts

EXTRACT	NORM.	2D HOUR	4TH HOUR
Injected 2 cc. kidney and spleen extract depancreatized dogs (Fisher)	0.125	0.126	0.126
Injected 10 cc. same extract as above	0.100	0.105	0.102
Injected 2 cc. kidney, spleen and muscle of depancreatized dogs (Fisher)	0.094	0.098	0.099
Injected 10 cc. same extract as above	0.109	0.114	0.112

the spleen of normal dogs and obtained no rise but a decided fall in the blood sugar value of starved rabbits (table 2), using first 2 cc. and later 8 cc., the latter brought the blood sugar down to 0.065 but we were unable to put the animal in convulsions, as the rabbit must have a blood

sugar value of 0.034 to 0.049 to produce this effect. This method of preparation has been adhered to during the remainder of the investigation. We next tried an extract of kidney and obtained positive results (table 2), but as the rabbit looked markedly depressed at the end of the nine and one-half hours, the blood sugar value was determined and to our astonishment the lowest reading was recorded at this point. The animal was accidentally fed while the blood sugar value was being calculated so we immediately repeated the experiment. A rabbit was starved for twenty-four hours and then injected with 2 cc. of the same extract as that used above and a twenty-hour sample of blood drawn (table 2), the blood sugar had started to return to normal at this point but was still very low. The experiment was now repeated using 8 cc. of the same preparation and a twenty-four-hour sample determined (table 2), a low value was again recorded. Next we decided to try a preparation of muscle from normal dogs and our results (table 2) checked very well with those obtained with the spleen preparations, failing to have the lasting effect of the kidney preparations.

Having isolated an insulin-like substance from normal tissues, we then prepared an extract from the spleens and kidneys of five depancreatized dogs, five days after the operation. We injected 2 cc. of this extract into starved rabbits and found no drop in the blood sugar (table 3) but rather a slight rise. This experiment was repeated using 8 cc. of the same extract and there was a slightly higher rise in the blood sugar noted, but no subsequent fall. In order to check these results we extracted the spleens, kidneys and muscles of two more depancreatized dogs and obtained similar results (table 3).

Discussion. The preceding data would tend to indicate that there is an insulin-like substance in the tissues, that this is not an inherent part of the cell but rather the hormone secreted by the cells of the islets of Langerhans and carried to and stored in the various tissues, by way of the blood stream.

When Banting and Best first discovered insulin it seemed probable that it could be isolated from the various tissues of the body if any procedure could be perfected to isolate the active substance in the small amount in which it is present in the tissues. The first method used by the above investigators was not delicate enough to detect it, as both their and our results proved negative when this method was used and to this fact we ascribe their failure.

The Murlin method also was not delicate enough for the isolation of the active fraction or if it was, the slight positive results were masked by the preliminary rise in the blood sugar value, due to the toxic fraction in the extract.

Somewhat better results were obtained with the Shaffer method and as soon as we tried Fisher's modification of this method it proved beyond a doubt that such a blood sugar lowering substance exists in at least some of the tissues.

The first precipitation ("toxic fraction") acts in every manner similar to the corresponding fraction obtained from pancreas (6), namely, when injected into the abdominal cavity of a rat it induces tetanic convulsions (as shown by Fisher with the toxic part obtained from pancreas) within eight minutes. There was some temporary recovery but the animal continued hypersensitive and went into convulsions frequently. In sixteen minutes the animal usually died in coma. It is this fraction that was the probable cause of our primary rise in blood sugar value, for these symptoms were never noted after we found a means of removing this substance from the extract.

As soon as we obtained positive results, we immediately tried to put rabbits into hypoglycemic convulsions, in order to have a means of standardization of the extract and in this way to compare our product quantitatively with pancreatic insulin but although we doubled and tripled the dosage we were never able to produce this degree of hypoglycemia notwithstanding the fact that 0.047 mgm. was reached, a point very near the level which normally produces convulsions. Whether this shows that there is some toxic material left in the preparation, or whether our extract is not true insulin but rather an insulin-like body of a slightly different nature, we are not prepared to say until more work has been done on this subject.

Our most important results appeared to be with the kidney insulin, because at that time Collip (2) had not yet reported the prolonged hypoglycemia by yeast extracts, and in nearly all research on insulin so far reported it had been found, on subcutaneous injections, that the lowest point in the blood sugar value was about the end of the fourth hour and that the value had returned to normal at the sixth or seventh hour. When we discovered that the blood sugar was still low at the end of nine and one-half hours and at twenty and twenty-four hours (table 2) we were at a loss to explain this unless it is due to an altered structure of the molecule or a retarded absorption due to an acquired side chain. This fact may be of some clinical value, for if the blood sugar can be held down close to normal for twenty-hours or longer by a single subcutaneous injection, it would reduce the required number of injections in a patient at least one-half. More experimental work must be done on the kidney insulin before its true value can be determined.

Having isolated an insulin-like substance from the tissues of dogs and cattle, the question arose whether this substance is inherent in the tissues in general or merely due to the stored product of the pancreas. We

therefore depancreatized a series of five dogs and five days after the operation killed them and extracted their spleens and kidneys. This extract proved to be inactive as far as reducing the sugar content of the blood of normal rabbits. The experiment was repeated using muscle in addition to spleen and kidney and as this also proved negative we feel safe in assuming that the insulin-like substance in the organs is either stored pancreatic insulin or substance produced in the tissues under the influence of the pancreas.

CONCLUSIONS

1. A substance can be extracted from the kidney, the spleen, and the muscle of normal dogs and cattle, possessing an insulin-like action in reducing the blood sugar of rabbits.
2. This substance can be demonstrated only after removal of the "toxic fraction" in the extract.
3. The "toxic fraction" from kidney, spleen and muscle acts physiologically similar to that obtained from the pancreas, in the preparation of insulin by the Shaffer-Fisher method.
4. The insulin extract prepared from the kidney maintains hypoglycemia for a long period, twenty-four hours or more.
5. The insulin-like substance in these tissues disappears shortly after pancreatectomy.

The author wishes to express his thanks to Dr. A. J. Carlson, at whose suggestion this research was undertaken, for his helpful criticism during the progress of this work, and for the revision of the manuscript.

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TEMPERATURE VARIATIONS IN RABBITS[1]

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The febrile reactions observed after injections of a great variety of heterogeneous substances which are known to contain protein, have often been ascribed to the protein constituents, but no direct evidence has so far been presented to support this assumption. Therefore, an attempt to determine the ultimate cause of the temperature phenomena in the so-called "protein fevers" is timely. However, before such a study could be made, it was found necessary to observe carefully the sensitiveness of the heat-regulating mechanism of the animal chosen for the investigation. This paper accordingly is introductory to a larger study of the so-called "protein fevers" presented in a later paper in this JOURNAL.

The determination of normal temperature variations in man or animals is an essential pre-requisite to the study of the effect of any factor upon the heat-regulating mechanism of the animal body. Such temperature studies have been made frequently upon rabbits and guinea pigs since these animals in particular have been the subjects for non-specific fever and anaphylaxis investigations. In this paper the temperature of rabbits only is considered.

The variations as well as the average level of temperatures reported by numerous investigators differ widely. It is well known that the heat-regulating mechanism of a rabbit is very susceptible to external influences and that moderate exercise, food, rough handling, etc., will cause a rise in temperature of several tenths of a degree. Therefore the differences in the technic employed by the different investigators in handling and keeping the animals may explain their different results.

The authors have attempted to standarize these conditions as a foundation for a later study of protein fevers. It is believed that the external influences, such as mentioned above, can be effectively minimized so that the normal variations in temperature will in no way mask those registering very delicate responses of the animal organism.

[1] The data in this paper are taken from the dissertation presented by Florence B. Seibert for the degree of Doctor of Philosophy, Yale University, 1923.

[2] Porter Fellow of the American Physiological Society, 1922–23.

Technic. The hourly temperatures of forty rabbits, practically all of which were males weighing 1 to 3 kilos, were determined throughout the day from about 8 a.m. to 6 p.m. The animals were kept in a light, well-ventilated room in small 1 × 1½ foot metabolism cages and every other week allowed to run at liberty for a few days in 3 × 7 foot pens. They were fed oats, water and cabbage or lettuce at 6 o'clock in the evening, after the last temperature readings were taken. Inasmuch as the experiments were performed in an isolated part of the building, there were practically no disturbing influences.

The temperatures were measured in the rectum with a standardized thermometer inserted beyond the sphincter for approximately two minutes.

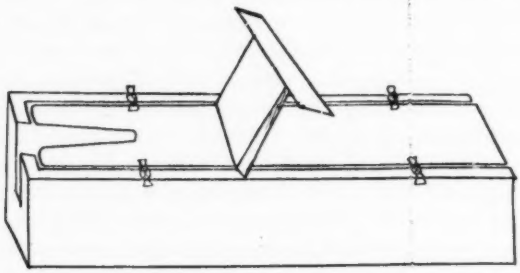

Fig. 1

The rabbits were gently lifted from the cage and held in a natural position on the lap. By paying special attention to the temperament of each animal it was possible to obtain temperatures hourly throughout the day without the slightest struggle on the part of any of the animals. At least one record of the normal daily temperature variation was obtained for every animal before it was subjected to an experiment.

The substances studied were injected into an ear vein, and the doses were so regulated that the volume of fluid used was 5 cc. in all cases. Two normal temperature readings were taken before the animal was injected in order to make sure that it was in the proper state of health. The rabbit was then placed in a box shown in the diagram so that it was in a natural position with its head held firmly by the lid of the box in order that it could be injected without the assistance of another person. The amount of struggle and the fright on the part of the animal was thus minimized.

The solutions were injected from a sterile syringe. In order to insure the sterility of the solutions, they were at first filtered through a Berkfeld candle into sterile flasks but later when only fresh solutions were used, filtration was found to be unnecessary and in fact often disadvantageous. The average dose of protein given was 5 mgm., and the method of preparing the solution was as follows:

Five-tenth gram of finely powdered protein was shaken until completely dissolved, with 10 cc. N/10 NaOH or $NaHCO_3$ made from freshly distilled water. This was diluted to 50 cc. with freshly distilled water, giving a 1 per cent solution. Ten cubic centimeters of this were then made to 100 cc. with freshly distilled water, giving a $\frac{1}{10}$ per cent solution. Five cubic centimeters, which were equivalent to 5 mgm. of the protein, and approximately 0.4 mgm. of the alkali were injected.

Experimental data. The average temperature of forty rabbits was 39.05°C., and this includes temperatures recorded in all seasons of the year except the summer. This average is slightly lower than that reported by some other investigators. For example, the average temperature stated by Richet (4) was 39.55°C.; Bock (1), 40°C.; Frothingham and Minot (2), 39.9°C.; Moore (3) 39.68°C. Pembrey in 1890 reported 38.7°C. as his average.

The average daily variation—that is the range from the lowest to the highest temperature registered during the day by the forty rabbits—was 0.54°C. with a probable error of ± 0.15°C. Representative temperature curves are found in the accompanying chart. Moore (3) has shown that it is possible to reduce this variation to practically zero by placing the rabbit in a 6 × 8 inch box with its head in a special holder. But such precautions were found to be unnecessary in the studies for which this work was undertaken as a control, since the variation obtained when a fever was produced was found to be two to three times as great as the normal variations. Although the average normal daily temperature variation in rabbits is only 0.54°C.—no more than is found in man—yet it is evident that the time of the day at which the temperature reaches its maximum or minimum is not the same from day to day and herein lies the difficulty in the study of fever by this method. Therefore, a febrile reaction, to be designated as such, must be accompanied by a temperature variation which clearly falls outside of this normal range and must have distinctive characteristics which would unquestionably place it in a different category from the normal variations.

Analysis of "positive" reaction. The character of the temperature curve of a positive reaction following the injection of certain agents is such as to distinguish it from a normal curve. For example, as shown in the chart, within one to three or four hours after the injection of a substance the temperature reaches a maximum which is considerably higher than any maximum point found on a normal curve. Within five to six hours the temperature has generally again reached normal. Consequently such a physiological response may be recognized by the rather sharp and steep rise in the curve shortly after the injection of the agent. Moreover, a negative reaction following the injection of a substance may be distinguished from the positive reaction since its curve is unlike the fever curve but is similar to the normal curve. This also is shown in the chart. Only three

curves of each group are given but these are typical of all; *1*, the normal curves, *2*, the negative reaction curves, and *3*, the positive reaction curves of over 700 experiments performed on the forty rabbits.

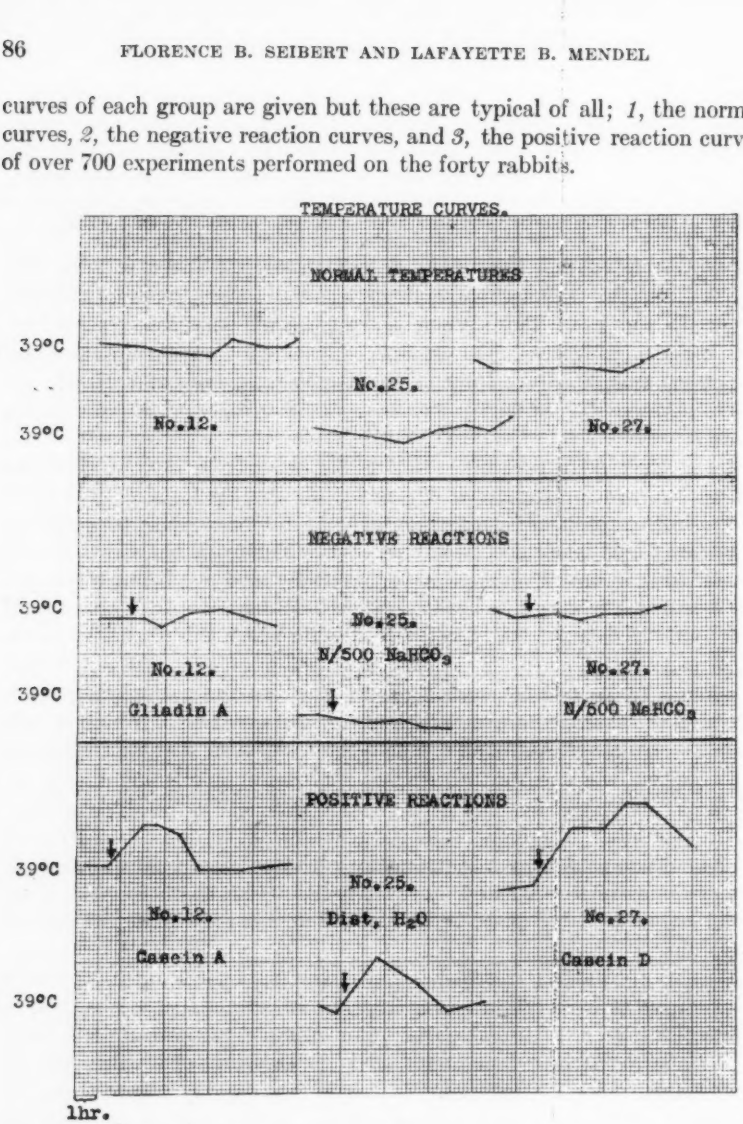

Chart A. A square on the ordinate = 1°C.; on abscissae = 2 hours.
The number of each animal is found with its curve.
The arrows indicate the time at which the injection was made. Since the curves are plotted from points at which the temperatures were recorded, the arrow sometimes erroneously appears to be on an ascending portion of the curve.

That the identification of a positive physiological reaction upon the basis of the character of its temperature curve is justifiable may be seen from the following table. Approximately 450 experiments were grouped as "positive" or "negative" solely from the character of their curves. The temperature ranges for the corresponding experiments were then calculated and it was found that the average daily variation for positive reactions varied between 1.04°C. and 1.40°C. while that for the negative reactions ranged from 0.44°C. to 0.67°C., which is approximately the same as the normal variation of 0.54°C.

TABLE 1

SUBSTANCE INJECTED	POSITIVE REACTION		NEGATIVE REACTION	
	Number experiments	Temperature variation	Number experiments	Temperature variation
		°C.		°C.
Water	69	1.06	143	0.48
Other solvents	19	1.04	60	0.47
Protein solutions	93	1.40	37	0.44
Miscellaneous	6	1.40	17	0.67
Hydrolysis products of proteins	9	1.40	6	0.65

From this it is clear that the average temperature variation accompanying what we have called a positive reaction is more than twice as much as the average normal variation and is therefore something tangible and useful in the study of fever production. Furthermore, the method employed for determining the normal temperature of rabbits is adequate and sufficiently delicate for the study of fevers.

SUMMARY AND CONCLUSIONS

Rabbits can be successively used for the study of fevers produced by the injection of water, proteins and other agents.

Analysis of the normal temperature variations of forty rabbits shows the average temperature to be 39.05°C. with a normal daily variation of 0.54°C. under standardized conditions.

A febrile reaction following the injection of a substance in rabbits is easily recognized by the character of the temperature curve, and by the fact that the average temperature variation in such a case is much greater than the normal variation or the variation found in a negative reaction of the same rabbit.

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PROTOCOLS

Normal temperatures of rabbits

NUMBER OF RABBIT	NUMBER OF DAY EXPERIMENTS	AVERAGE TEMPERATURE	AVERAGE DAILY VARIATION
		°C.	°C.
2	2	39.98	0.37
6	2	38.78	0.60
7	4	39.21	0.37
8	4	39.56	0.43
9	2	39.57	0.30
10	2	39.02	0.40
11	1	39.00	0.25
12	1	39.03	0.35
13	2	39.08	0.30
14	5	39.04	0.62
15	1	39.01	0.70
16	1	39.00	0.70
17	1	38.87	0.75
18	2	39.37	0.57
19	1	39.62	0.70
20	1	39.17	0.30
21	1	39.18	0.30
22	1	39.13	0.50
23	2	39.28	0.90
24	1	38.93	0.75
25	1	39.13	0.60
26	1	39.12	0.90
27	1	38.60	0.40
28	1	39.02	1.00
29	1	39.45	0.30
30	5	39.09	0.94
31	2	39.11	0.35
32	1	38.92	0.60
33	2	38.84	0.65
34	1	38.90	1.20
35	1	39.07	0.40
36	1	39.23	0.50
37	1	39.15	0.30
38	1	38.90	0.40
39	1	38.91	0.20
40	1	38.63	0.50
41	1	38.81	0.70
42	1	38.32	0.90
43	1	38.46	0.40
44	1	38.62	0.20
Average...40	63	39.05	0.54

Specimen protocols for the chart

RABBIT 12		RABBIT 25		RABBIT 27	
Time	Temperature	Time	Temperature	Time	Temperature
		Normal temperature			
	°C.		°C.		°C.
9:00 a.m.	39.1	8:25 a.m.	39.15	7:50 a.m.	38.7
10:45	39.0	11:55	38.9	8:45	38.5
11:40	38.9	12:50	38.8	11:30	38.5
2:00 p.m.	38.8	2:20 p.m.	39.1	12:45	38.5
3:00	39.2	3:35	39.2	2:35 p.m.	38.4
4:30	39.0	4:40	39.1	3:35	38.7
5:15	39.0	5:35	39.4	4:40	38.9
5:45	39.2	6:30	39.4		
		Negative reactions			
9:00 a.m.	38.8	7:55 a.m.	38.6	8:55 a.m.	39.0
10:30	Injected	8:56	38.6	10:02	38.8
11:00	38.8	9:35	Injected	10:38	Injected
11:50	38.6	11:00	38.4	11:42	38.9
1:00 p.m.	38.9	12:35	38.5	12:55	38.8
2:30	39.0	1:42 p.m.	38.3	1:50 p.m.	38.9
3:55	38.8	3:07	38.3	3:35	38.9
5:00	38.7			4:43	39.1
Gliadin A injected		N/500 $NaHCO_3$ injected		N/500 $NaHCO_3$ injected	
		Positive reactions			
8:15 a.m.	39.2	9:11 a.m.	39.0	9:15 a.m.	38.6
9:15	39.2	9:46	38.8	10:45	38.7
9:30	Injected	10:15	Injected	11:06	Injected
11:00	40.1	11:42	40.1	12:30	40.0
11:30	40.1	1:25 p.m.	39.5	2:00 p.m.	40.0
12:30	39.9	2:45	38.9	3:00	40.6
1:30 p.m.	39.1	4:30	39.1	3:58	40.6
3:30	39.1			6:15	39.6
5:45	39.2				
Casein A injected		Dist. H_2O injected		Casein D injected	

FEVER-PRODUCING SUBSTANCE FOUND IN SOME DISTILLED WATERS[1]

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Emphasis must again be placed upon the fact that distilled water kept under ordinary conditions is not necessarily as pure and as physiologically harmless as is usually supposed. The importance of a careful study of the fever-producing properties of water is apparent, since it is the universal solvent and must therefore be considered when conclusions are drawn concerning protein, salt and salvarsan fevers, etc. As a result of the following work it can be seen that one is not able in any hospital or research laboratory to take for granted that a physiological saline made by dissolving C.P. salt in distilled water taken from a tank and then autoclaving, is physiologically inert.

Historical. As far back as 1865 Billroth (2) reported in some cases a fall and in others a rise in temperature following the intravenous injection of distilled water into dogs. Bergmann (1) mentioned similar results. Later the numerous salt fevers and salvarsan fevers were thought to be specific types of fever until Wechselmann (12) pointed out that if the salvarsan solutions were carefully made with freshly distilled germ-free water, no reaction symptoms occurred. He postulated that the febrile reaction so frequently reported by former investigators was due to contamination products of the solutions and not to the salvarsan itself. Müller (10) proceeded to find bacteria in distilled water and gave a method for their detection.

Inspired by this work and following it, Hort and Penfold presented most comprehensive and logically planned investigations, in which they clarify the assumption that the toxicity of the salvarsan as well as of other solutions was due to contamination products of the water. Their work cannot be over-emphasized since, in spite of its clearness, exactness and fundamental value, it has been neglected by most succeeding investigators, especially in the field of protein fever. Although Hort and Penfold (7) clearly showed that water, sterile, chemically pure, and injected immediately after distillation will not produce a fever, they still leave open the question as to what the occasionally found fever-producing substance is, where it comes from, and how it develops. They called it a pyrogenic substance, showed that it develops on standing, is filterable, and suggested that it is of bacterial origin. That the toxicity is not due to the intact bacteria themselves

[1] The data in this paper are taken in part from the dissertation presented by Florence B. Seibert for the degree of Doctor of Philosophy, Yale University, 1923.

[2] Porter Fellow of the American Physiological Society, 1922-23.

was substantiated by the fact that the injection of the centrifugalized sediment containing the bodies of the bacteria did not produce a fever. They further pointed out that the microörganisms can be grouped into two classes with regard to the kind of fever produced by them; one a late continuous fever and the other an immediate fugitive fever, which closely resembles the fever produced by the injection of some waters. As a result of their work they attribute the various types of fever, such as water, salt, sugar, ferment, tissue, anesthesia, surgical, sea-water and salvarsan fevers to a common cause—a pyrogenic substance, so far unidentified.

Other theories than that a bacterial pyrogenic substance is the cause of non-specific fever have been presented. Some of the most outstanding of these involve the doctrine of specific ion effect, hemolysis, hydrogen-ion concentration, etc. These will be more fully discussed together with their chief exponents under the corresponding items in the section on experimental data. More emphasis is not given to them since the author believes the evidence for their support is scanty and in most cases can be excluded.

EXPERIMENTAL DATA. *Technic.* All experiments were performed in duplicate at least. The intravenous route for the injection of substances was used exclusively and 5 cc. amounts were always injected unless stated otherwise. The experiments were performed upon rabbits. The justification for the selection of this animal for temperature studies, the recognition of a "positive" febrile reaction and its distinction from normal variations in temperature, as well as the technic employed, are all fully described in a previous paper (see p. 83) presented in this JOURNAL.

Possible causes of fever. It has been clearly shown that the handling of the rabbits necessary to the determination of hourly temperatures produces variations in temperature throughout the day, at most one-half to one-third as great as those found after the injection of typical fever-producing agents. Moreover, the examination of the character of the temperature curve is an adequate and useful method of detecting fevers.

Other factors than the manipulation of the animal were studied. For example, the *rate of injection* of the water does not play an important rôle in fever production. One sample of water when injected quickly into two rabbits—that is, at the rate of ten and twelve seconds—induced fever as soon and to the same extent as when it was injected into two other rabbits during periods of eighty and one hundred and ten seconds respectively. Similarly, a "negative" water was injected both quickly and slowly, and in each case the reaction was negative. A third lot of water gave positive results after both slow and fast injections.

As mentioned in a previous paper, solutions were at first sterilized before injection by *filtration through a Berkefeld candle.* This method was chosen since protein solutions were to be used and heating was undesirable. It was then conceived that a fever-producing substance might sometimes be washed off of the Berkefeld candle and thus render the material tested falsely "positive." After filtering a protein solution the candle was always

removed and thoroughly scrubbed with a brush under running water. N/10 sodium hydroxide and distilled water were then repeatedly filtered through it and it was again boiled for five minutes in order to sterilize it for the next filtration. But even this repeated washing proved insufficient in some cases. At eight different times with solutions such as water, sodium chloride, sodium hydroxide and sodium bicarbonate, a fever was produced and found to be due to contamination by the Berkefeld candle. In each case it was demonstrated that the same solution which after filtration through the Berkefeld candle produced a fever in a rabbit, did not do so when some of the same solution was injected unfiltered. Therefore, when possible it is desirable not to use this method of sterilizing; or it is necessary first to filter through the candle freshly distilled water, and if this does not give a fever, then to filter the substance under examination.

This observation raised the question whether or not it is necessary to sterilize protein, water or salt solutions if they are used fresh. One water produced a positive reaction whether it was filtered or not and three lots of water gave no fever both before and after filtration. Therefore, it can be seen that filtration, provided the Berkefeld candle is clean, makes no difference in the reaction produced by the solution injected. In other words, *the fever-producing substance is filterable*, and it can not be removed in this manner.

Another factor for consideration is the constancy or the production of a reaction by a substance injected several times into the same animal as well as the effect of the first injection into different animals. How far the *hypersensitiveness or immunity* of an individual animal may enter into the results must be clearly defined. It is well known that different species react differently and also that healthy animals do not react the same as diseased ones of the same species. Consequently, very slight differences in the degree of response of different animals are to be expected. In the first place all experiments were made in duplicate at least, and in some cases as many as twelve animals were used for the same experiment. Where the two animals did not respond similarly, further experiments were performed in order to determine whether the cause of disagreement lay in the animal, the technic used, or the agent injected. Surprisingly consistent results were obtained throughout the entire investigation and, where there was no error in the technic, all animals responded similarly to the injection of the same substance.

From the following table it will be seen that large numbers of experiments performed either on the same day or on different days upon a great many animals with the same lot of water gave identical results. For example, in the case of water lot I six experiments performed on four different rabbits and on three different days, all gave positive reactions. On the contrary, with lot IX, in sixteen experiments, on eleven different rabbits and on three

different days, all the reactions were negative. Therefore it would seem that the resistance of the individual animals is not the determining factor in fever-production, but rather that the reactions are dependent upon the nature of the agent injected.

TABLE 1

NUMBER OF LOT OF WATER	NUMBER OF EXPERIMENTS	NUMBER OF RABBITS USED	NUMBER OF DAYS FOR EXPERIMENTS	KIND OF TEMPERATURE REACTION
I	6	4	3	+
II	5	4	2	+
III	4	4	1	−
IV	9	7	2	−
V	4	4	1	−
VI	8	7	3	+
IX	16	11	3	−
X	9	6	4	−
XI	10	10	1	+
XII	12	12	1	−
	29	13	5	−
XIII	4	4	2	−
XVI	4	4	1	+
XVII	6	6	2	−
XVIII	12	9	3	+

TABLE 2

NUMBER OF THE RABBIT INJECTED	NUMBER OF INJECTIONS WITH WATER IN EACH ANIMAL	TIME IN DAYS FROM FIRST TO LAST INJECTION
18	15	2½
21	14	2
22	16	3
23	16	2½
24	19	4½
25	24	4½
26	20	3½
27	11	3
28	17	4½
29	7	1
30	8	5
31	14	4
32	16	4
33	9	3
34	5	1½
35	3	½
36	2	1½
38	3	3½
39	2	3

Furthermore, the question is important whether or not *previous injection* of an animal either with the same substance or with any other non-specific

agent effects its response. Krehl (9) emphasized the fact that animals previously injected gave a greater rise in temperature than after the first injection. This statement is contrary to the findings of the author. From the data of over two hundred experiments with distilled waters injected into nineteen rabbits, either on successive days or at long intervals, there was never any indication of an immunizing or of a sensitizing effect. The production of a reaction depended upon the water injected rather than upon the number of previous injections or upon the animal used. Table 2 contains a summarized statement of these data.

One rabbit injected upon four successive days with the same water reacted practically to the same extent each day. Further evidence of the absence of an influence due to previous injections will be presented in another paper in this journal which records that proteins, especially casein, have been repeatedly injected without indication of anaphylaxis. Such results may be due to the fact, which is known, that it is particularly difficult to obtain anaphylaxis in the rabbit.

That *hemolysis* of the blood might be the cause of fever was first suggested by Freund (6) and this theory has had many followers, among whom is Yamakami (15). He claimed that the intensity of the reaction was increased depending upon the amount of water injected. He reported that the water he used was freshly distilled and that less than 5 cc. gave no febrile reponse while with 40 to 60 cc. there was a maxium reaction and with above 70 cc. there was a fall in temperature. In answer to this, Penfold and Robertson (11) repeated the experiments exercising most elaborate care in the preparation of their solutions to insure sterility and non-contamination; and they were not able to reach the same conclusions. Hemolysis was produced, as evidenced by the hemoglobinuria, but in no case was fever elicited with amounts of water as large as the largest used by Yamakami. They reëmphasized the importance of the pyrogenic substance probably of bacterial origin in the waters which gave fevers. The author of this paper also undertook an experiment on this phase of the problem, the results of which are evidence against hemolysis as a cause of fever.

In two rabbits about 1 cc. of blood was withdrawn from the ear vein into a syringe containing a few cubic centimeters of inactive water. The blood was thoroughly hemolyzed by shaking and then reinjected. Another syringe was quickly transferred to the needle and enough more physiological saline was injected in order to make a total of 5 cc. of liquid. In neither case was there a temperature rise.

In another experiment the injection of as much as 20 cc. of water gave no fever in the rabbit.

Therefore hemolysis may be neglected as a factor in the reactions studied in this paper.

Much consideration in recent years has been paid in physiology to the influence of the *hydrogen ion concentration* of solutions. That this factor may be influential in the production of fever was recently emphasized in a paper by Williams and Swett (14), in which they claim that saline and glucose solutions more acid than a pH of 6.5 produce chills and prostration in patients, while if such solutions are brought to the pH of blood no reaction is said to follow their administration. In order to determine whether or not the results of the present investigation were affected by

TABLE 3

"POSITIVE"		"NEGATIVE"	
Lot number of water	pH	pH	Lot number of water
III + CO_2	<5.2	4.6	XI sec. frac.
		5.2	XI autoclaved
VII	5.9	5.8	XVII sec. frac.
I first frac.	6.0	6.0	I sec. frac.
II	6.2	6.2	XXVI
XXI	6.6	6.6	XII sec. frac.
I	6.8	6.8	V + glass
XXV tap	7.0	7.0	XXII
XVIII tap	7.3	7.2	XXVII
XIII residue	7.7	8.0	IX
III residue	>8.0	>8.0	XIX

TABLE 4

WATER		OVALBUMIN		CASEIN A	
pH	Reactions	pH	Reactions	pH	Reactions
4.6	− −	4.6	+ +	6.6	+
7.0	− −	6.5	+ +	7.1	+
8.0	− −	8.0	+ +	7.3	+

the hydrogen-ion concentration of the solutions, the pH of every solution injected was determined colorimetrically. The following table shows that it has been possible from the extensive data obtained to select and list under either positive or negative reactions, results with different lots of water with a hydrogen ion concentration anywhere within a range from more acid than pH 5.2 to considerably more alkaline than pH 8.0.

Table 4 further illustrates the unimportance of the hydrogen ion concentration of the solutions, in that the same lot of water, the same lot of ovalbumin, and the same lot of casein gave consistent results whether the pH of the solution corresponded to that of the blood or was more acid or more alkaline.

With the elimination of factors of technic, of individual resistances and immunities of the animals themselves, of hemolytic factors, and of hydrogen-

ion concentration considerations as fever-producing elements, there remained only the obvious question as to whether or not the fever of distilled water was due to contamination. Impurities of all kinds were sought for. Bock's (3) doctrine of *specific ion* effect was considered. He claimed that the Na-ion was able to produce fever, while Wideroe (13) argued for the chloride and bromide ions. Hort and Penfold, on the other hand, showed that sodium chloride solutions up to 25 per cent concentration made from freshly distilled water did not produce fever. The author also has evidence against a specific ion effect. Sodium chloride solutions in concentrations of 0.9 per cent, 8 per cent and 10 per cent gave no fever, and in one case as much as 20 cc. of the 0.9 per cent solution were without effect. Solutions of ammonium chloride, ammonium sulfate, sodium hydroxide, sodium bicarbonate and hydrochloric acid in the concentrations in which they were used as solvents gave no reactions, when they were made up in freshly distilled water. Moreover, a buffer solution, made as described by Fleisch (5) except that freshly distilled water was used throughout, produced no fever in six different rabbits. This solution contained a variety of ions, such as Na, K, Ca, Mg, Cl and PO_4 with a pH of about 6.8. Glucose solutions also when similarly prepared gave no fever. Furthermore, that the pyrogenic substance is not of a character or present in sufficient quantity to be visible in the spectrum is evident from a negative spectroscopic study of a "positive" water.

An impurity which readily occurs to mind is *glass*. Nothing but pyrex apparatus was used throughout and the flasks used for the water were kept for that purpose only. It is hard to conceive of dissolved glass being the important factor when one water will or will not give a fever even when kept in many different flasks and, on the other hand, some waters when put into a certain flask will give fevers while others in the same flask do not. One water which had been standing in a soft glass bottle for years still gave no fever even though it most likely contained some glass in solution since the pH of the water was 7.2. Furthermore, glass wool was placed in freshly distilled water and although some of the glass went into solution as seen by the increase in the pH number, no reaction was obtained.

At first corks covered with tin-foil were used but later this metal was discarded in order to eliminate any inorganic ions from that source. No difference in the results was noticed. A *cork extract* made by boiling cork in freshly distilled water gave only a slight fever in proportion to the deep color of the solution. It therefore scarcely seems likely that any of the high fevers reported are due to this source.

The ordinary *laboratory gases* such as CO_2, O_2, H_2S and HNO_3 fumes, which were suspected at one time or other to be causes of the development of a pyrogenic property of water, were studied, but no direct evidence was found for attributing fever producing properties to them.

The one remaining impurity to be considered, of course, is that of a *bacterial nature*. While no water which was kept sterile produced a fever on standing, it is also true that some non-sterile waters after standing as long as five months still were non-pyrogenic. Different evidence, therefore, must be presented before one can claim that bacterial contamination as such is the cause of fever. The author believes, however, that such evidence is given in this paper. The method of experimentation by which this conclusion was reached is briefly as follows: first, a careful study of the *nature and properties* of the pyrogenic substance was made, and then *isolation and identification* of a pyrogen-producing bacterium was attempted.

All waters do not produce fever on injection. Tap waters drawn in different seasons of the year, for example in January, February, June and November, when tested always caused fever. Distilled waters taken from different tanks and containers about the laboratory showed a variability in this respect. From six lots of water so chosen, three proved to be fever-producing and three non-reactive. Therefore, ordinary distilled water can not be relied upon to be safe for fever studies, when it is used as a solvent for injection of substances.

Following such a conclusion it became imperative to outline *a method for the preparation of a non-pyrogenic water*. As has been intimated by numerous former investigators, water injected when freshly distilled will not produce a fever. The present investigation firmly supports this statement. On fifty-three different occasions water was distilled, using a thoroughly clean, all-pyrex apparatus, and injected within twenty-four hours thereafter; and in not a single instance did the distillate produce fever. Moreover, nine different waters which had been shown to produce fever, were distilled, and the distillate in all cases was non-fever-producing. It was found necessary to use a trap above the distillation flask in order to prevent the pyrogen from being mechanically carried over into the distillate. The connecting stoppers were rubber which had been cooked several times in alkali and then in distilled water in the usual manner. At first they were covered with tin-foil but this was eliminated in order to avoid any metallic contamination. It was not found necessary to sterilize the distillate since the latter was used soon after the distillation.

The distillate in some instances was sterilized immediately and set away for a month, and in one case it was sterilized, sealed, and then put aside for five months. When again tested the waters were still without fever-producing effect. Therefore, it would seem that the important step in preserving a good water rests in the immediate sterilization after distillation. As short a time as four days is sufficient for the pyrogen to develop in a distillate when it is kept under clean but non-sterile conditions. For example, five lots of water which had been shown to give no fever,

developed a pyrogenic substance, when left to stand in the laboratory for four or five days. On the other hand, there were eight lots which still remained good after thirteen to forty-one days and even five months. One lot which had been standing for several years in a glass-stoppered bottle was still non-pyrogenic. This development of a fever-producing substance in non-sterile waters simulates the development of an organism.

However, if the substance is of bacterial origin, it nevertheless is not identical with the bacterial bodies themselves, since filtration through a Berkefeld candle does not render a pyrogenic water non-fever-producing. This fact was emphasized previously when factors of technic were being eliminated as causes of fever. It would seem as though the pyrogen is a *filterable product* of bacterial origin.

If the pyrogen is a bacterial toxin, it would be expected to be *heat-labile*. This was found to be true, although at first sight it did not appear to be the case. Seven lots of pyrogenic water were distilled and the first distillate fraction, second distillate fraction, and residue of each were tested

TABLE 5

NUMBER OF THE LOT OF WATER	ORIGINAL WATER REACTIONS	FIRST FRACTION REACTIONS	SECOND FRACTION REACTIONS	RESIDUE REACTIONS
I	+ +	+ +	− −	
II	+	+	−	
III	+ +	− −	− −	+ +
XI	+ + +	− −	− −	+ +
XIII	+ +	− −	− −	+ +
XVIII	+ +	− −	− −	− −
XXVII	+ + + +	−		+ + + +

as shown in table 5. In four cases the fever-producing substance was found to remain in the residue. However, in case of lots I and II, the pyrogen was found in the first distillate fraction and seemed therefore to be of a volatile nature; but subsequent experiments showed the error to lie in the fact that a trap had not been used in the distillation and some pyrogenic substance was carried over mechanically when boiling first commenced. Although this amount of boiling as well as autoclaving at fifteen pounds pressure for twenty minutes did not completely destroy the pyrogen, it seemed probable that some destruction took place, since all the distillate fractions as well as the residue of water XVIII were non-pyrogenic while the original water itself gave a fever.

Further evidence for the destruction of pyrogen by heat is presented in the following experiment. Lot XXVII was distilled to one-fourth its original volume and 1, 3, 5 and 10 cc. amounts of the original water as well as of the residue after distillation were injected. Where the amount for injection was less than 5 cc. sufficient freshly distilled water was added to

make the solution to this volume. The results can be seen on chart A. The reactions produced by the original water were greater in degree than those produced by the corresponding amounts of the residue. Therefore, there is a destruction of the pyrogen which takes place faster than its

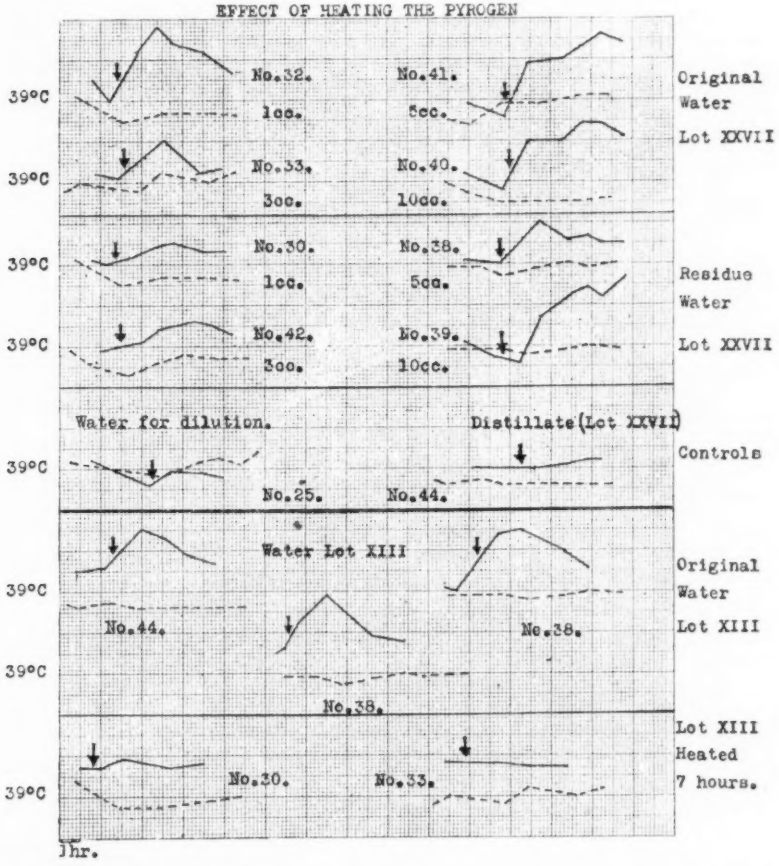

Chart A. The dotted lines represent normal temperature curves. The number of each animal is found with its curves. The arrows indicate the time at which the injections were made.

concentration. The natural question arises as to the possibility of complete destruction by heat. In answer to this, lot XIII which was demonstrated to be pyrogenic was rendered completely non-pyrogenic by refluxing for seven hours. Therefore, the toxic substance in water can be destroyed completely by heat, but long drastic heating is necessary.

All the evidence so far presented indicates that the pyrogen in distilled water is a filterable, heat-labile, non-volatile, fever-producing product of bacterial origin. The following experiments by which this theory seems to be proved will be given in some detail.

A water was freshly distilled and the distillate divided into two parts. One part was merely preserved in a corked flask. The other part was "seeded" with one drop a water (lot I) which had become pyrogenic on standing. Both parts were injected into rabbits and gave negative results, showing first that the water used was non-

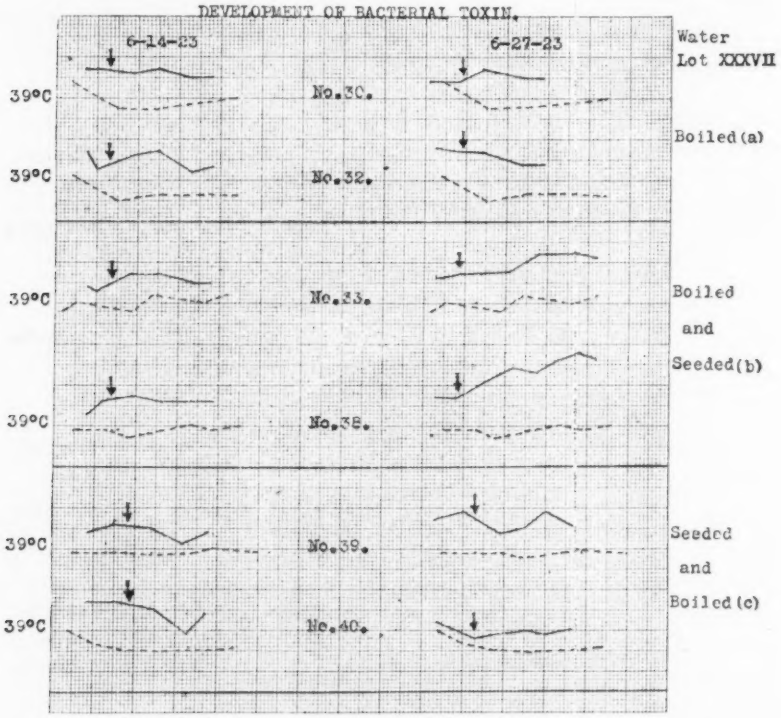

Chart B

pyrogenic, and secondly, that the one drop of "positive" water used for seeding was not sufficient in itself to make the water pyrogenic. After standing one week both parts were again tested and the one which had not been seeded was "negative," while the "seeded" water was very "positive."

This shows that mere inoculation is sufficient to make a water pyrogenic after it has stood several days and clearly points to the fact that growth of some organism is the essential factor to the development of the toxicity of the water.

A more carefully planned and conclusive experiment was instituted. For this see charts B and C.

A water was redistilled and the distillate divided into three parts. Portion *a* was boiled in order to sterilize it; portion *b* was boiled, cooled, and then "seeded"

IDENTIFICATION OF THE TOXIN

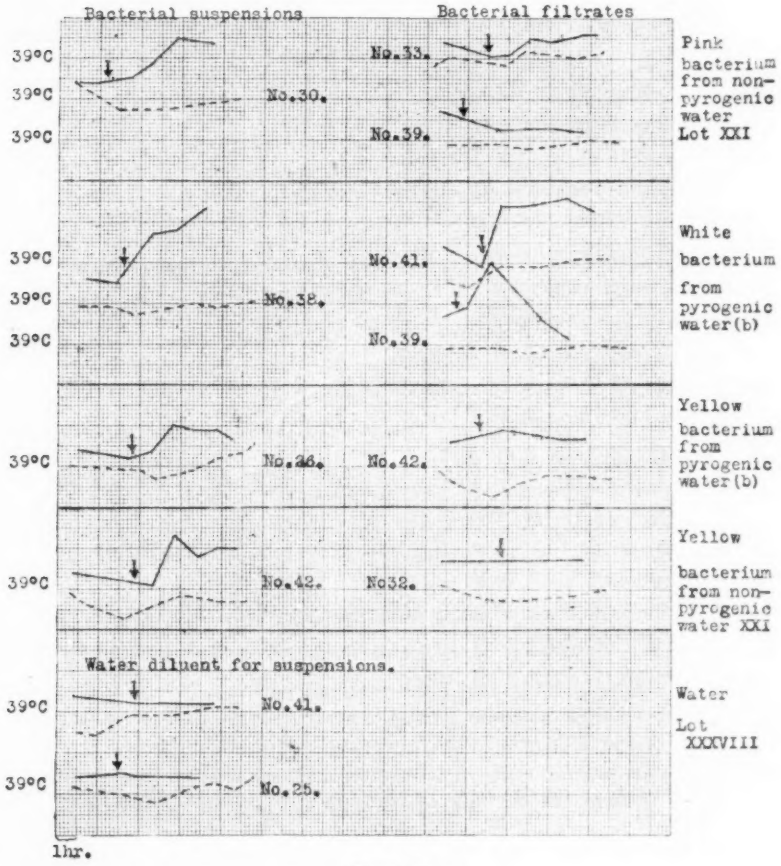

Chart C

with a drop of the water described in the previous experiment as becoming pyrogenic on standing due to "seeding" with a pyrogenic water; portion *c* was "seeded" with a drop of the same water and then boiled to kill any living bacteria present. All three portions tested immediately gave no fever, but after standing two weeks portion *b* only gave a fever.

This substantiates the bacterial nature of the contaminating substance.

The three portions were then plated on agar and *a* and *c* were sterile as was to be expected, while *b* contained 122,000 colonies per cubic centimeter. These colonies were predominately one type, the description of which will be given later. From the colony which predominated, a pure agar culture was made and then a loopful of the bacteria was suspended in freshly distilled inactive water until the solution was

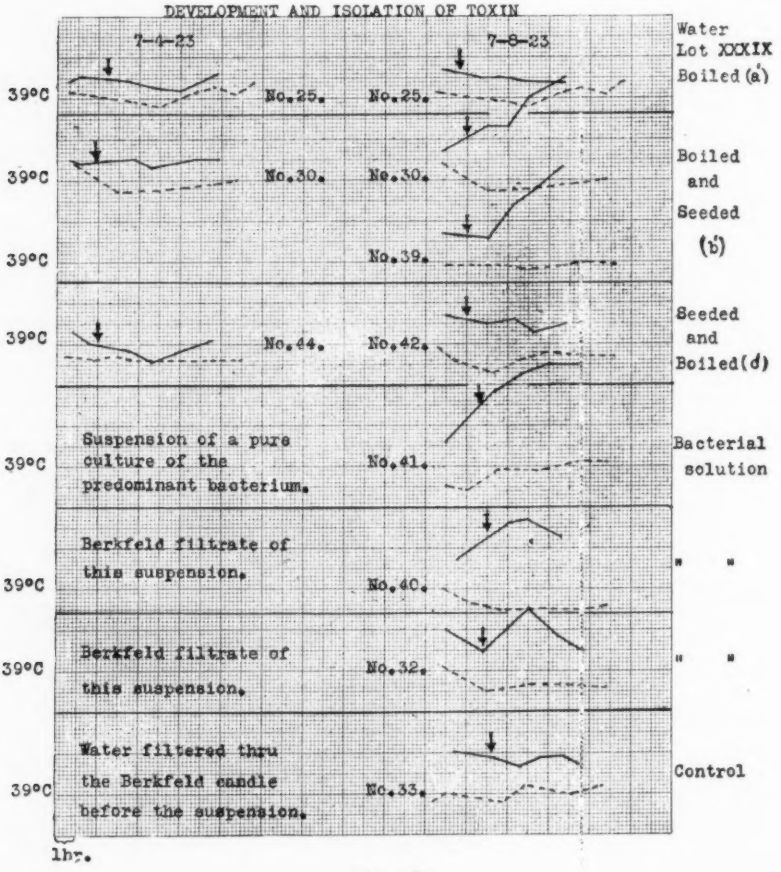

Chart D

turbid. This solution, containing the live bacteria, when injected, produced a fever. In order to test out the theory that it is a bacterial product which gives the fever, the bacterial bodies were filtered off by means of the Berkefeld candle and the filtrate injected. A fever was produced and this fever was undoubtedly due to a filterable substance rather than to contamination by the candle, since freshly distilled water filtered first was not rendered pyrogenic. Moreover, the bacterial filtrate was sterile when plated.

Therefore, a filterable product produced by this characteristic bacterium appears without doubt to be the cause of the fever.

Starting with a fresh distillate, the entire experiment was repeated and each step unquestionably confirmed. (See chart D.)

Further evidence that the fever is produced by a product of this specific bacterium rather than of bacteria in general is presented in the following experiments. The same characteristic white colony also predominated when two other pyrogenic waters were plated. However, there were two pyrogenic waters which were found to be sterile, and it is possible in these cases that the pyrogen remained after the bacteria themselves died.

On the other hand 42,000 colonies per cubic centimeter were counted in a water which had remained non-pyrogenic in spite of the fact that it had been standing for five and a half months under non-sterile conditions. The predominant colony, however, in this case was pink. The fact that the number of colonies was about twice as great as the 25 to 26,000 colonies per cubic centimeter found in the two pyrogenic waters just mentioned indicates that it is not so much a matter of numbers of bacteria as of type of bacteria. Pure cultures were made of this pink bacterium as well as from three other types of colonies isolated from both the pyrogenic and non-pyrogenic waters. Fevers were obtained with suspensions of all of these bacteria but in no case with the Berkefeld filtrates, as shown in chart C. In each case water was filtered first through the Berkefeld candle and shown to be non-pyrogenic before the bacterial solutions were filtered. These controls are not recorded on the chart. Therefore, a fever-producing substance is not formed by any of the bacteria isolated except by the white one which predominated in "positive" waters, and this would indicate it to be the bacterium which produces the pyrogen in water.

An attempt was made to identify this bacterium and the following properties were found.

It lives in distilled water and develops a fever-producing filterable pyrogen.
Agar colony—white, transparent, glistening, round, smooth colony which yellows on standing and darkens toward the center. It develops slowly at 20° and 30°C., and is granular under the microscope.
Agar slant—white, transparent, soft, glistening, spreading, somewhat beaded and ropy.
Broth culture—good growth, develops turbidity and white scum on surface.
Litmus milk—reduction of litmus, slight acidity, rennet-like curd, slight digestion after two weeks.
Sugars—salicin, mannit, arabinose, inosite, maltose, dextrose, lactose, xylose, rhamnose, saccharose—growth and considerable alkalinity in all with a development of pH of 8.6. The highest alkalinity appeared in arabinose and xylose.
No gas formation in dextrose and lactose.
Spore test—negative.
Capsule test—Welch's method on agar culture—positive.
Motility—positive.
Morphology—very small, thin, rod-like bacillus, Gram negative.

SUMMARY AND CONCLUSIONS

Fevers may be produced in rabbits by the intravenous injection of distilled water as it is usually found in laboratories.

The fever is not caused by the manipulation of the animals necessary to the determination of hourly temperatures. It is not dependent upon the rate of the injection of the water, nor is it due to a contaminating substance from a Berkefeld candle. The resistance and hypersensitiveness of the individual animals, as well as the influence of previous injections, do not determine the fever.

Hemolysis of the blood and hydrogen ion concentration of the solutions higher or lower than that of the blood are not the causes of fever.

Impurities, such as inorganic salts, glass dissolved from the container, gases from the air, constituents of the cork stopper have all been excluded as probable factors in fever.

Water can be assured to be non-pyrogenic by distilling it through an all-pyrex apparatus containing a spray-catching trap and then using it within twenty-four hours.

A study of the nature and properties of the pyrogenic substance showed it to be present in some but not all distilled waters.

It may develop in a water in four to five days, is filterable through a Berkefeld candle, and is destroyed by long drastic heating only.

The pyrogen is a filterable product produced by a specific bacterium some of the properties of which are given. Filtrates fom other bacteria isolated from both pyrogenic and non-pyrogenic waters did not give fever.

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PROTEIN FEVERS[1]

With Special Reference to Casein

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Although the blood always contains a variety of proteins as normal constituents, the introduction of a foreign substance of this class into the circulation either by direct injection into the blood stream or by some other parenteral path whereby digestive changes are avoided, is likely to bring about a physiological reaction in the organism. The response may be mild and undetectable except by very refined methods; or it may vary in degree from a barely perceptible fever to severe shock symptoms, involving fever, chill, dyspnea, paralysis, and even death. Such a so-called non-specific reaction is sometimes elicited by the injection of bacterial suspensions, vaccines, serums, milk, etc., and is of great physiological interest. Moreover, it is the basis of a type of therapy which has been used very extensively during the last few years. The many beneficial effects reported in diseases which failed to respond to other methods of treatment have given this therapy practical value in the medical world, and make its scientific study particularly interesting and worthwhile at the present time.

Arthritis especially is said to have been quite successfully treated by the injection of such non-specific agents as typhoid vaccine or milk. Cecil (6), for example, reported that 40 per cent of the cases of rheumatic and toxic arthritides recovered completely in two to ten days as a result of the effects of typhoid vaccine injections. Alhswede (1) described a favorable influence upon acute and chronic staphylococcus infections of the skin and upon gonorrheal complications, from the injection of germ-free milk solutions. Culver (8) used gonococcus protein, and Dehio (9) used deuteroalbumoses with success in gonorrheal infections. Certain cases of eye disease have yielded excellent results by treatment with milk injections. The above mentioned cases serve merely as examples of the many diseases which have been reported to be favorably affected by this mysterious "protein therapy."

[1] The data in this paper are taken from the dissertation presented by Florence B. Seibert for the degree of Doctor of Philosophy, Yale University, 1923.

[2] Porter Fellow of the American Physiological Society, 1922-23.

As has been pointed out, many different and apparently unrelated agents—vaccines, serums, milk, etc.—may favorably affect the same disease, and on the other hand, one substance may cause improvement in many different diseases. Therefore, the symptoms which are more or less manifested in all of these cases are properly spoken of as the non-specific reaction. Until the work of Roemer (18) in 1891, a viewpoint of strict specificity was held and suggestions contrary to this idea were swept aside without consideration by the medical world. But when this investigator reported marked effects upon tuberculous animals with injections of pneumobacilli and bacillus pyocyaneus, and when Rumpf (19) almost at the same time presented favorable results in a series of typhoid patients treated with subcutaneous injections of pyocyaneus vaccine, the existence of a non-specific therapeutic effect became not only possible but even promising.

The symptoms of this non-specific reaction are manifold, but all of them are not manifested in every case. The chill is one of the most characteristic symptoms to be noticed and it may set in as soon as fifteen minutes after the injection or as late as eight to ten hours thereafter, depending upon the agent used. Following the chill, there is generally a marked febrile reaction, the temperature rising in man sometimes to as high as 103° to 104°F. and reaching its maximum in from three to four hours after the injection. The occurrence of this maximum may be considerably postponed when certain agents are used, especially by the intramuscular route. Coincident with the increase in temperature there is an increase in pulse rate and blood pressure. Shortly after this, profuse sweating commences and the accompanying peripheral dilatation reduces the blood pressure and pulse rate. Other symptoms, such as nausea, vomiting, headache, feeling of malaise, herpes, diuresis and albuminuria may or may not be experienced. In the severe shock reaction a fall in temperature, violent dyspnea, abdominal collapse, paralysis and even death may occur. The metabolism is increased as evidenced by the increased nitrogen excretion. The permeability of the capillaries is increased for a short time. As might be expected, there is an alteration in the blood picture. The erythrocytes, blood platelets, sugar, fibrinogen, thrombokinase and leucocytes have all been observed to increase. The variations of the leucocytes in particular have been extensively studied by many investigators who have usually detected a leucopenia after the injection, followed in turn by a leucocytosis. Moreover, the albumin-globulin ratio of blood proteins is found to be disturbed. On the day after these symptoms are experienced a general feeling of euphoria sets in and gradually the patients improve until in many cases a cure is effected.

Whether or not the discomfort of such a general reaction is necessary to the achievement of the remarkable therapeutic effects reported is an

important question—one as yet unanswered. According to Brooks and Stanton (5) unpleasant symptoms are not essential. They observed improvement although there were no systemic reactions in cases of arthritis treated with the "lower fractions" of digestion products of ox fibrin. However, the great majority of investigators and clinicians lay much emphasis upon the necessity of a sharp general reaction in order to obtain satisfactory therapeutic results.

Assuming therefore the desirability of such a reaction, a careful study of the individual symptoms as well as of the factors producing the symptoms is essential. In this investigation the febrile reaction has been chosen as the specific symptom for study, since the heat regulation of an organism is extremely delicate and very susceptible of quantitative measurement, and also since its disturbance is one of the earliest appearing and most prevalent symptoms following the injection of foreign protein.

How the heat regulating mechanism of the body is maintained or disturbed is an elusive and much debated question among physiologists, but that it is upset when certain foreign substances are introduced into the blood stream, is a recognized fact. Non-specific therapy is particularly concerned with the nature of these disturbing substances and with the conditions under which they cause the disturbance.

Historical. Influential factors were first defined by Krehl (12) in a remarkably comprehensive study of many unrelated substances that were able to produce fever. He emphasized the importance of species, the number of previous injections an animal has received, and the dosage and mode of administration. Moreover, the state of health of the individual animal is of considerable importance in determining the extent to which the reaction is manifested. But most important of all is the substance itself which is injected.

Petersen (17) cites such agents as blood serums, proteins, protein split-products, enzymes, tissue extracts, vaccines, bacterial extracts, colloidal metals, yeasts, salt and sugar solutions, formulin, solusin, hetol, iodids, turpentine, antipyretics, and even distilled water. Although they would appear at first sight to be unrelated, yet the question arises whether or not it will be possible to find a chemical nucleus or physical property common to all of them, which is capable of upsetting the heat regulating mechanism.

Since the time when Kühne (13) found by analysis that the tuberculins, then so extensively used, consisted largely of protein cleavage-products, and since Krehl (12) obtained febrile responses with a most heterogeneous variety of substances many of which did not contain protein, the question of whether or not the cause of the fever is protein has been a fundamental one. The problem today remains almost in its original form. It is true that the study of milk therapy has disclosed some interesting evidence but even this is most fragmentary.

The first serious investigation with milk used as a fever-producing agent was undertaken by Besredka (4) in 1909, but not until 1916 was it used as a satisfactory therapeutic agent, when Schmidt (23) and Saxl (21) began to report favorable clinical results. From then on numerous investigators have used milk with marked success. Many have suggested that the casein in the milk produces the beneficial effects, but few have attempted to test purified casein. Lindig (14), however, made casein

from milk and injecting this in sodium bicarbonate solution produced in patients with septic, gonorrheal or tubercular diseases a chill and headache, a marked rise in temperature after the third injection, and then decided improvement. Similar results have been reported by Zimmer (34), Arweiler (3), Taege (25) and Voehl (29). But to other investigators it appeared that casein may not be the important therapeutic factor in the milk. Müller (16), for example, prepared a solution of milk free from casein and still obtained a reaction. Therefore he concluded that endotoxins from the bacteria in the milk were the cause of the rise in temperature rather than the casein itself. Similarly, Uddgren (26) and Ryhmer (20) believed the fever must be due to bacterial products, since with fresh sterile milk they obtained scarcely any reaction. Furthermore, Aman (2) laid emphasis upon the importance of the "antagonistic colloidal activity" of the salts in the milk, and made a preparation "albusol" which was said to be milk freed from bacterial products and salts and rendered neutral to litmus. This gave no reaction.

Besides casein very few other proteins have been investigated with this question in mind. von den Velden (30) mentioned using ovalbumin and serumalbumin with very little success. With the exception of these few isolated cases little attempt has been made to determine whether or not the protein itself is the agent which is able to produce a fever in an animal.

The fact that similar fevers were being reported following the injection of salt solutions and salvarsan solutions gave this entire problem a broader aspect and made the question more fundamental. For example, Schaps (22) reported temperature fluctuations in children following the injection of as little as 1 cc. of physiological saline, grape sugar, and lactose. Englander (10) claimed to have cured typhoid patients in fourteen to sixteen days by the intravenous injection of salt solutions. On the other hand, other investigators such as Weiland (32) and Wechselmann (31) were reporting the absence of febrile reactions with saline, sugar and salvarsan solutions.

A review of an earlier paper on "Fever producing substance found in some distilled waters," which is presented in this JOURNAL, will show clearly that the fevers obtained with sugar and the various salt solutions as well as with water are due to a bacterial contamination of the solution. Is it not possible that this same contamination adheres to the protein under examination, rendering it fever-producing? With this point in mind, it was the purpose of the present investigation to study the effect produced by a variety of more or less purified proteins and in addition, of some individual proteins highly purified. The fact that a protein cannot be inactivated by purification will not necessarily mean that the reaction is not due to contamination rather than to the protein as such, since there may be a strong affinity between protein and the contaminating substance. Furthermore, the possibility of denaturizing the protein molecule in the process of purification must be carefully considered. With the premise that the fevers here reported are not due to any individual reactions on the parts of the animals themselves; or to any of the factors in the technic such as rate of injection, method of filtration and manipulation of the animals; or to hemolytic factors, or to the hydrogen ion concentration of the solutions, or to contaminating impurities such as dissolved gases or glass,

specific inorganic ions, cork extracts; or to the common solvent water if properly prepared,—an attempt was made to study the fever-producing power of pure native proteins.

Technic. The rabbit was used throughout and the technic employed has been fully described in an earlier paper on "Temperature Variations in Rabbits," presented in this JOURNAL. In every case the protein was dissolved with freshly distilled water and the solvent also was injected into two animals as a control experiment on the same day on which the protein was studied. If this test was not negative, the experiment was repeated. Some of the proteins studied were prepared at the Connecticut Experiment Station and were given through the courtesy of Dr. T. B. Osborne and Dr. H. B. Vickery, to whom thanks are expressed; the others were prepared by one of the authors.

Experimental data. The following proteins in 5 mgm. doses, except the majority of the alcohol-soluble proteins, gave a marked positive reaction in two animals each. From table 1 it can be seen that two lots of wheat gliadin, made in different laboratories and one lot of hordein produced no fever in rabbits. Both zein and rye gliadin gave only one slightly positive reaction out of five experiments. With millet seed protein one negative and one positive reaction were obtained and the alcohol soluble protein from milk, described recently by Wells and Osborne (33) gave only positive reactions. The fact that negative reactions were found only in the alcohol-soluble proteins points to a fundamental significance attached to the method of preparation or to a group property, and adds evidence to results reported later.

Casein, however, is the protein which has received the most attention in this investigation, since it is the one which is concerned largely in milk- and casein therapy. So far no one has reported the preparation of a pure casein which will give a negative reaction when injected into rabbits. But the authors have deemed it advisable to determine whether or not such a preparation could be made.

Casein was prepared according to Van Slyke's method by precipitating with acid the protein at its isoelectric point during rapid stirring. It was found necessary to separate the protein by centrifugation and then it was dried with 70 per cent and 95 per cent alcohol and finally with ether. The analysis showed: ash = 0.86 per cent, moisture = 8.45 per cent, fat = 2.02 per cent.

This casein preparation A, dissolved in sodium bicarbonate, gave a fever when injected in 50, 5 or 1 mgm. doses, and practically the same amount of temperature rise in all three cases. This fact cannot be without significance and makes doubtful the importance of the casein itself as the fever-producing element. All of the experiments with the various casein preparations are summarized in table 2. It is interesting to note that through-

TABLE 1

DATE OF EXPERIMENT	PROTEIN INJECTED	TEMPERATURE VARIATION +	TEMPERATURE VARIATION −	NUMBER OF ANIMAL	SOLVENT USED	pH OF SOLUTION
		°C.	°C.			
1–26–23	Squash seed globulin	1.6	—	28	10 per cent NaCl	6.3
	Squash seed globulin	0.9	—	33	10 per cent NaCl	6.3
1–26–23	Blue lupin globulin	1.4	—	24	10 per cent NaCl	6.3
	Blue lupin globulin	1.3	—	32	10 per cent NaCl	6.3
1–29–23	Cotton seed globulin	2.5	—	22	N/500 NaOH	>8.0
	Cotton seed globulin	1.4	—	33	N/500 NaOH	>8.0
1–29–23	Phaseolin	1.3	—	26	N/500 NaOH	>8.0
	Phaseolin	1.4	—	31	N/500 NaOH	>8.0
1–29–23	Lactalbumin	1.4	—	24	N/500 NaOH	>8.0
	Lactalbumin	1.0	—	32	N/500 NaOH	>8.0
1–29–23	Amandin	2.4	—	27	N/500 NaOH	>8.0
	Amandin	2.1	—	36	N/500 NaOH	>8.0
2– 2–23	Glycinin-soy bean	2.7	—	27	N/500 NaOH	>8.0
	Glycinin-soy bean	2.7	—	38	N/500 NaOH	>8.0
2– 2–23	Excelsin	1.5	—	26	N/500 NaOH	>8.0
	Excelsin	2.3	—	30	N/500 NaOH	>8.0
2– 2–23	Maize glutelin	1.7	—	28	N/500 NaOH	>8.0
	Maize glutelin	1.2	—	24	N/500 NaOH	>8.0
2– 2–23	Cannabin	0.6	—	31	N/500 NaOH	>8.0
	Cannabin	2.2	—	22	N/500 NaOH	>8.0
2– 2–23	Legumelin-vetch	1.4	—	33	N/500 NaOH	>8.0
	Legumelin-vetch	2.7	—	23	N/500 NaOH	>8.0
1–26–23	Conglutin	0.8	—	25	10 per cent NaCl	5.3
	Conglutin	0.8	—	36	10 per cent NaCl	5.3
6– 1–22	Gliadin-wheat A	—	0.35	12	N/50 NaOH	>8.0
	Gliadin-wheat A	—	0.35	13	N/50 NaOH	>8.0
12–15–22	Gliadin-wheat B	—	0.6	25	N/50 NaOH	>8.0
	Gliadin-wheat B	—	0.3	27	N/50 NaOH	>8.0
	Gliadin-wheat B	—	0.4	28	N/50 NaOH	>8.0
	Gliadin-wheat B	—	0.1	31	N/50 NaOH	>8.0
2–22–23	Hordein	—	0.2	30	N/500 NaOH	>8.0
	Hordein	—	0.5	40	N/500 NaOH	>8.0
2–15–23	Zein	—	0.5	36	N/500 NaOH	>8.0
	Zein	0.6	—	24	N/500 NaOH	>8.0
3–16–23	Zein	—	0.4	36	N/500 NaOH	>8.0
	Zein	—	0.6	38	N/500 NaOH	>8.0
	Zein	—	0.6	39	N/500 NaOH	>8.0
2– 2–23	Millet seed	—	0.3	24	N/500 NaOH	>8.0
	Millet seed	0.7	—	28	N/500 NaOH	>8.0
2–22–23	Gliadin-rye	—	0.4	36	N/500 NaOH	>8.0
	Gliadin-rye	0.9	—	39	N/500 NaOH	>8.0
3–16–23	Gliadin-rye	—	0.5	40	N/500 NaOH	>8.0
	Gliadin-rye	—	0.6	28	N/500 NaOH	>8.0
	Gliadin-rye	—	0.3	22	N/500 NaOH	>8.0
2–22–23	Milk-alcohol-soluble	0.9	—	32	N/500 NaOH	>8.0
	Milk-alcohol-soluble	1.3	—	38	N/500 NaOH	>8.0

TABLE 2

DATE OF EXPERIMENT	LOT OF CASEIN	AMOUNT OF REACTION		NUMBER OF ANIMAL USED	SOLVENT	pH
		+	−			
		°C.	°C.			
5- 9-22	Casein A—50 mgm.	1.45	—	9	N/50 $NaHCO_3$	—
11	Casein A— 5 mgm.	1.95	—	8	N/500 $NaHCO_3$	7.3
16	Casein A— 1 mgm.	1.15	—	12	N/2500 $NaHCO_3$	6.7
20	Casein A— 1 mgm.	1.25	—	12	N/2500 $NaHCO_3$	6.6
9-27-22	Casein A— 1 mgm.	1.70	—	15	N/2500 $NaHCO_3$	7.1
12- 5-22	Casein B—5 mgm.	0.80	—	18	N/500 $NaHCO_3$	6.9
	Casein B	1.00	—	27	N/500 $NaHCO_3$	6.9
	Casein B	1.40	—	34	N/500 $NaHCO_3$	6.9
12-12-22	Casein B	1.40	—	23	N/500 $NaHCO_3$	7.2
	Casein B	1.60	—	24	N/500 $NaHCO_3$	7.2
	Casein B	1.90	—	34	N/500 $NaHCO_3$	7.2
1- 5-23	Casein C—5 mgm.	—	0.60	26	N/500 $NaHCO_3$	7.7
	Casein C	—	0.30	33	N/500 $NaHCO_3$	7.7
	Casein C	—	0.40	35	N/500 $NaHCO_3$	7.7
	Casein C	1.50	—	28	N/500 NaOH	>8.0
	Casein C	1.20	—	32	N/500 NaOH	>8.0
	Casein C	1.60	—	36	N/500 NaOH	>8.0
1-30-23	Casein C	—	0.50	30	N/500 $NaHCO_3$	7.2
	Casein C	0.90	—	38	N/500 $NaHCO_3$	7.2
2- 3-23	Casein C	1.30	—	32	N/500 $NaHCO_3$	7.1
	Casein C	1.00	—	25	N/500 $NaHCO_3$	7.1
	Casein C	1.20	—	36	N/500 $NaHCO_3$	7.1
	Casein C	2.00	—	39	N/500 $NaHCO_3$	7.1
1-17-23	Casein D—5 mgm.	1.20	—	22	N/500 $NaHCO_3$	7.1
	Casein D	0.50?	—	26	N/500 $NaHCO_3$	7.1
	Casein D	1.00	—	24	N/500 NaOH	>8.0
	Casein D	1.10	—	31	N/500 NaOH	>8.0
1-30-23	Casein D	—	0.60	24	N/500 $NaHCO_3$	7.1
	Casein D	—	0.10	31	N/500 $NaHCO_3$	7.1
1-30-23	Casein E—5 mgm.	—	0.60	28	N/500 $NaHCO_3$	7.1
	Casein E	1.80	—	23	N/500 $NaHCO_3$	7.1
1-30-23	Casein F—5 mgm.	1.30	—	22	N/500 $NaHCO_3$	7.0
	Casein F	—	0.20	27	N/500 $NaHCO_3$	7.0
2-19-23	Casein F	0.50?	—	36	N/500 $NaHCO_3$	7.1
	Casein F	0.90	—	27	N/500 $NaHCO_3$	7.1
2-19-23	Casein G—5 mgm.	0.70	—	38	N/500 $NaHCO_3$	7.1
	Casein G	1.10	—	39	N/500 $NaHCO_3$	7.1

TABLE 2—*Concluded*

DATE OF EXPERIMENT	LOT OF CASEIN	AMOUNT OF REACTION		NUMBER OF ANIMAL USED	SOLVENT	pH
		+	−			
		°C.	°C.			
5–30–23	Casein G	1.10	−	30	N/500 $NaHCO_3$	7.2
	Casein G	0.90	−	25	N/500 $NaHCO_3$	7.2
	Casein G	−	0.50?	33	N/500 $NaHCO_3$	7.2
	Casein G	−	0.20	32	N/500 $NaHCO_3$	7.2
3–23–23	Casein H	−	0.20	41	N/500 $NaHCO_3$	6.5
	Casein H	−	0.20	38	N/500 $NaHCO_3$	6.5
	Casein H	−	0.20	39	N/500 $NaHCO_3$	6.5
	Casein H	−	0.40	22	N/500 $NaHCO_3$	6.5
	Casein H	−	0.20	36	N/500 $NaHCO_3$	6.5
	Casein H	−	0.20	40	N/500 $NaHCO_3$	6.5
4– 9–23	Casein H	−	0.30	32	N/500 $NaHCO_3$	6.8
	Casein H	−	0.50	39	N/500 $NaHCO_3$	6.8
3–30–23	Casein I	−	0.20	26	N/500 $NaHCO_3$	7.5
	Casein I	−	0.70	28	N/500 $NaHCO_3$	7.5
	Casein I	−	0.30	40	N/500 $NaHCO_3$	7.5
	Casein I	−	0.30	41	N/500 $NaHCO_3$	7.5
	Casein I	−	0.40	33	N/500 $NaHCO_3$	7.5
6– 5–23	Casein I	1.10	−	40	N/500 $NaHCO_3$	7.3
	Casein I	1.50	−	42	N/500 $NaHCO_3$	7.3
	Casein I	1.00	−	26	N/500 $NaHCO_3$	7.3
4– 9–23	Casein J	0.90	−	22	N/500 $NaHCO_3$	7.1
	Casein J	1.50	−	24	N/500 $NaHCO_3$	7.1
	Casein J	1.20	−	41	N/500 $NaHCO_3$	7.1
	Casein J	0.80	−	38	N/500 $NaHCO_3$	7.1
	Casein J	−	0.50	28	N/500 $NaHCO_3$	7.1
6– 5–23	Casein K	1.00	−	30	N/500 $NaHCO_3$	7.1
	Casein K	0.90	−	39	N/500 $NaHCO_3$	7.1
	Casein K	1.10	−	44	N/500 $NaHCO_3$	7.1

out all the experiments with casein there was never any indication of anaphylaxis or immunity due to former injections into the same animal.

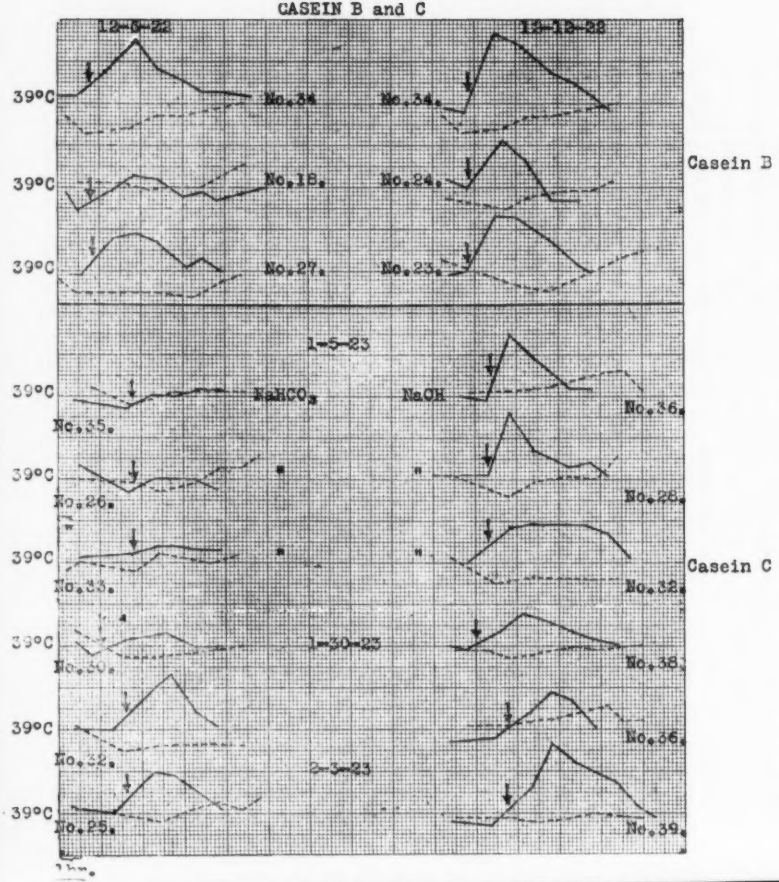
Various types of purification were studied in order to determine whether the febrile reaction was due to the protein itself or to a contamination product of the protein. For example, casein A was redissolved in alkali and reprecipitated with acid five times but still gave a fever when injected. As seen from the analyses—ash = 3.38 per cent, moisture = 4.39 per cent, fat = 0.2 per cent, and nitrogen = 14.7 per cent—casein B was practically fat-free and this would indicate that fat as an impurity is not the cause of fever.

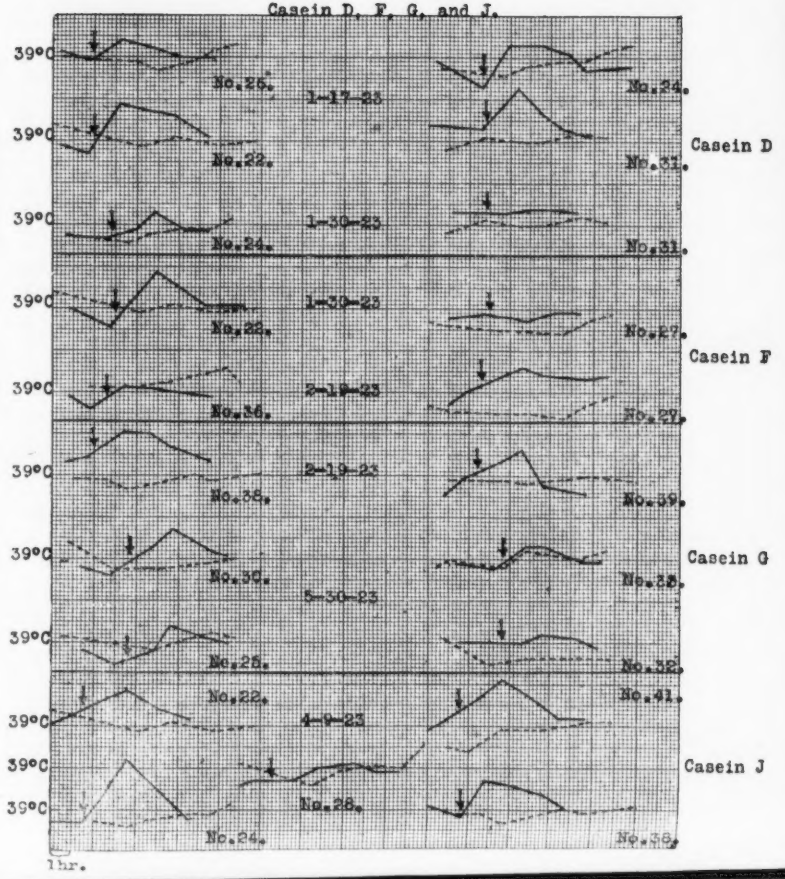
Casein B was then freed of salts by Loeb's (15) method—that is, by washing it six times with water, the pH of which was 4.6 or the same as the isoelectric point of casein. Each time it was thoroughly mixed with the water by grinding in a mortar for five to ten minutes, then filtered and finally dried by means of 70 per cent and 95 per cent alcohol, and ether. The isoelectric water used was made from freshly distilled water and was shown in two tests on rabbits to have no fever-producing power. This lot of casein C was therefore very low in ash—0.4 per cent—and might possibly have contained less pyrogenic contamination by virtue of its being washed with inactive water. The results with this preparation were the most promising so far obtained. For example, when dissolved in $NaHCO_3$, as in all previous experiments, and injected, it gave no temperature rise. However, in order to test the effect of a different solvent the casein was dissolved in NaOH but in this case fever was obtained. Twenty-five days later the same lot of casein, dissolved in $NaHCO_3$, was tested and this time gave a fever in one rabbit but not in another. Four days after this, four rabbits responded with a fever. This apparent development of a fever-producing property in an originally inactive casein is significant and is further evidence against the importance of the casein itself.

An attempt to repeat the experiment by preparing another lot of ash-free casein D with the same technic, however, was unsuccessful. Even greater care was employed in the preparation of this casein and Cohn's (7) method of redissolving at a pH of 7.0 in order to eliminate casein in combination with divalent ions was used. It was precipitated then at a pH of 4.7, washed with water at a pH of 4.7, and this process repeated six times. The ash content was only 0.1 per cent but nevertheless febrile reactions were obtained, indicating that the salt impurities are not the important factors. Forty-four days later this casein dissolved in $NaHCO_3$ gave two negative reactions. The apparent inactivation of the casein with time is unexplainable and, of course, directly opposed to the results obtained with the previous lot of casein.

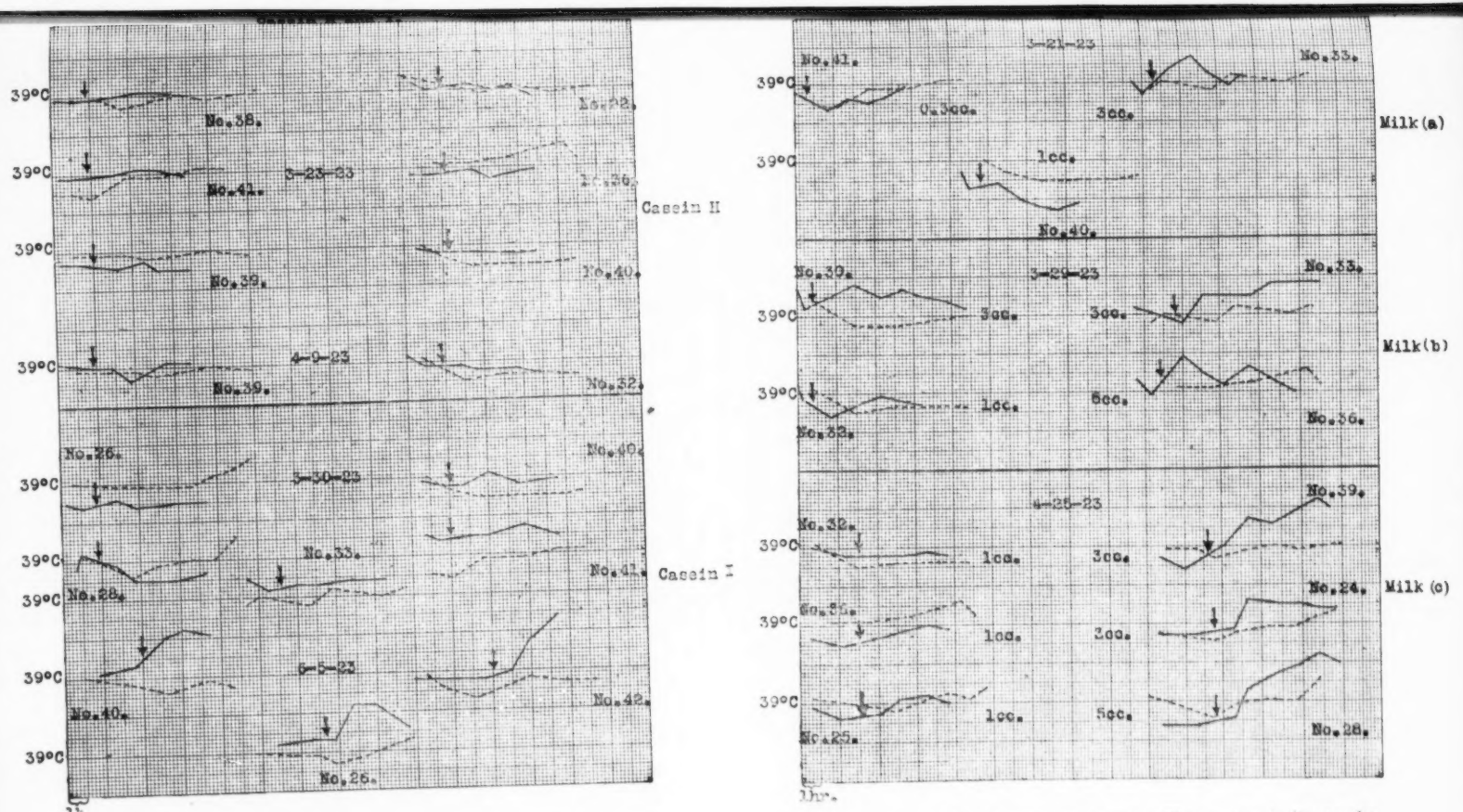
Two other samples of casein D and E, the latter of which was prepared at the Connecticut Experiment Station, gave negative and positive reactions.

Another type of purification suggested by Vaughan's (28) work in which he ascribed the toxic portion of Witte "peptone." to the alcohol-soluble fraction was undertaken. For example, casein C was thoroughly washed by grinding in a mortar with absolute alcohol six times, but this preparation —casein G—still produced a fever. Extraction with hot alcohol did not prove any more efficacious in rendering casein inactive. Casein C after being boiled with 95 per cent alcohol three times for ten-minute periods and then being filtered each time still gave a fever—casein J.


CASEIN B and C
12-6-22
13-12-22
39°C
No.38.
No.34.
No.18.
No.24.
No.27.
No.23.
Casein B
1-5-23
NaHCO3
NaOH
No.35.
No.36.
No.26.
No.28.
No.33.
No.32.
Casein C
1-30-23
No.30.
No.38.
No.32.
No.36.
2-3-23
No.25.
No.39.
1hr.


Casein D, F, G, and J.
39°C
No.26.
1-17-23
No.34.
No.22.
Casein D
No.31.
No.24.
1-30-23
No.31.
No.22.
1-30-23
No.27.
Casein F
No.26.
2-19-23
No.27.
No.38.
2-19-23
No.39.
No.30.
Casein G
No.38.
3-30-23
No.26.
No.32.
No.22.
No.41.
4-9-23
Casein J
No.28.
No.24.
No.38.
1hr.


No.41.
3-21-23
No.33.
39°C



Charts A-D. The dotted lines represent normal temperature curves. The number of each animal is found with its curves. The arrows indicate the time at which the injections were made.

Therefore the fever-producing property of casein would not seem to be due to adhering fat, salts or an alcohol-soluble substance. But the fact that several negative reactions were obtained after certain types of purification led one to suspect that the fevers were due to a contaminating substance rather than to the casein itself. Since bacteria were the source of the pyrogen in water, may not this also be the case with casein? Therefore, instead of trying to remove a pyrogenic substance already present in the casein, attempts were made to prepare a casein in which bacterial contamination was prevented from occurring.

TABLE 3

DATE OF EXPERIMENT	LOT OF MILK	AMOUNT OF REACTION		NUMBER OF ANIMAL USED	AMOUNT INJECTED
		+	−		
		°C.	°C.		cc.
3-21-23	Milk *a* fresh	−	0.40	41	0.3
	Milk *a*	−	0.70	40	1.0
	Milk *a*	1.00	−	33	3.0
3-29-23	Milk *b* fresh	−	1.30	32	1.0
	Milk *b*	0.60	−	30	3.0
	Milk *b*	1.00	−	33	3.0
	Milk *b*	1.00	−	36	5.0
4-25-23	Milk *c* fresh	−	0.20	32	1.0
	Milk *c*	−	0.50	36	1.0
	Milk *c*	−	0.60	25	1.0
	Milk *c*	1.80	−	39	3.0
	Milk *c*	0.90	−	24	3.0
	Milk *c*	1.80	−	28	5.0

Milk *a* was obtained under as sterile conditions as possible. A healthy Jersey cow was led out doors, brushed, and the udder sterilized with surgical technic. The milker's hands were similarly sterilized. A pint of milk was discarded and then about a quart was milked into a sterile bottle. The milk was then brought to the laboratory within an hour. The authors wish to express appreciation to Miss Elizabeth Carey for obtaining the milk in the manner described.

A nutrient broth culture showed slight contamination with streptococcus lactis.

Three rabbits were injected with the milk *a*—in 0.3 cc., 1 cc. and 3 cc. doses respectively—and a slight fever was obtained only in the case of the 3 cc. dose. (See table 3.)

From the remainder of the milk, casein H was prepared as quickly as possible.

A dilute solution of hydrochloric acid was made by adding a few drops of concentrated HCl to a water which had just been freshly distilled and shown to be inactive when injected into a rabbit. With this acid the casein was precipitated

at its isoelectric point—pH 4.6—from the milk during constant stirring. The casein was then filtered off with suction, ground in a mortar several times with some of the freshly distilled water, the pH of which was 4.7 and filtered each time. Following this the casein was treated similarly twice with alcohol and then put into ether. The ether mixture was stood on a radiator and allowed to come to a boil, then filtered. This was repeated three more times and then the casein was treated repeatedly with cold ether and finally dried. Although the milk was rich in fat the casein prepared from it was a fine white powder and probably did not contain much fat.

Two days afterward, a 0.1 per cent sodium bicarbonate solution of this casein H was prepared in the usual way and injected. Six rabbits gave absolutely negative results. (See table 2.) Seventeen days later the same casein was tested and still found to be negative.

On another day a sample of fresh sterile milk *b* was obtained with the same technic as described above, and when plated it was found to contain only 3000 to 4000 organisms per cubic centimeter. The injection of 1 cc. of this milk again failed to produce a reaction while 3 and 5 cc. doses did produce a fever. Casein I prepared immediately from this milk in the manner described for casein H and injected on the following day gave no reactions in five rabbits, thus confirming in detail the results obtained in the former experiment. However, when tested again in sixty-six days, positive reactions were obtained. The casein had been kept in a clean, but non-sterile, well-stoppered bottle and it is possible that bacterial contamination may be the cause of the development of a fever-producing property on standing.

However, if casein is prepared from milk in which there are relatively few bacteria and if it is made and tested before the latter have had time to produce a toxic substance in any quantity, no fever is elicited by the injection of the casein. The inactivity of the casein can scarcely be due to a denaturizing effect on the protein, since it was never in contact with acid or alkali beyond the concentration equivalent to its isoelectric point, nor was it subjected to drastic heat or cold at any time. When warmed with the ether the temperature naturally never rose above that of the boiling point of ether. Furthermore, a denaturization cannot be attributed to alcohol treatment since alcohol extraction did not inactivate caseins G and J.

Since non-fever-producing casein can be made from fresh milk and since the milk from which it was made nevertheless gave a fever when injected in doses larger than 1 cc., it was attempted to determine whether or not even this fever could be eliminated if the milk which was taken under sterile conditions was boiled immediately. Such boiling would kill the few bacteria present and would thus prevent a possible formation of the toxic substance before it reached the laboratory to be tested. Milk *c* treated in this way, however, gave the same results as the two previous samples—that is, fever with doses above 1 cc.

Therefore the following confirmed facts can be stated in regard to the fever-producing properties of milk. There is something in milk which produces a fever in rabbits in doses greater than 1 cc. This is not a bacterial product which develops in the milk in vitro. It is possible, however, that such a product may be formed in vivo since it is practically impossible to obtain sterile milk and some organisms live in the ducts of the mammary gland. From previous work it can be seen that the casein, sugar, salts and fat constituents of the milk are not responsible for the fever. But the many other components of the milk, such as proteins, other than casein, and possibly some unknown factors are yet to be studied.

The fact that casein could be successfully prepared from milk in such a manner as to be free from fever-producing properties and still not be denaturized, inspired an attempt to prepare egg proteins, edestin and hemoglobin similarly.

Ovalbumin crystallized in the usual way in nine different cases produced positive reactions, and this includes experiments in which the hydrogen ion concentration of the solution was varied from more acid than pH 5.2 to more alkaline than pH-8.0 If the purified protein made from egg white produces a fever, it might be expected that the egg white itself, if injected in a dose sufficient to contain an equivalent amount of ovalbumin, would also produce a fever. Such an experiment was performed.

A strictly fresh egg was sterilized according to Schottelius' (24) method. It was washed with freshly distilled water, which was also sterile. The shell was then pricked with a sterile needle and some of the egg white drawn into a syringe. One-tenth cubic centimeter of the egg white, equivalent to 12 mgm. of ovalbumin, was injected into the ear vein of a rabbit, and then another syringe was quickly transferred to the needle and 5 cc. physiological saline were injected. Two-tenths cubic centimeter, equivalent to 24 mgm. of ovalbumin, was injected in the same manner into another rabbit. Neither of these rabbits showed the slightest temperature fluctuation. (See table 4.)

This absence of a reaction with fresh, sterile egg white can scarcely be explained by assuming that the salts in the egg white inactivated the protein, since similar salts have been tested previously with and without other proteins and no indication of such a phenomenon has ever been detected. Hydrogen ion concentration cannot explain it on the basis of former experiments. Some change had unquestionably been produced in the protein in the process of isolation that had rendered it capable of disturbing the heat-regulating mechanism of the rabbit. In view of the experience with casein and with the pyrogenic substance in water, are we not justified in asking whether or not this same pyrogenic substance might be the ultimate cause of the fever-producing power of samples of crystallized ovalbumin?

TABLE 4

DATE OF EXPERIMENT	PROTEIN INJECTED	AMOUNT INJECTED	TEMPERATURE VARIATION		pH	NUMBER OF ANIMAL	SOLVENT
			+	−			
		mgm.	*°C.*	*°C.*			
12- 8-22	Crys. Ovalbumin	5	1.35	—	6.6	22	Water
	Crys. Ovalbumin	5	2.50	—	6.6	27	Water
	Crys. Ovalbumin	5	1.80	—	6.6	31	Water
2- 9-23	Crys. Ovalbumin	5	1.90	—	4.6	33	Water
	Crys. Ovalbumin	5	1.80	—	4.6	22	Water
	Crys. Ovalbumin	5	1.10	—	6.5	26	Water
	Crys. Ovalbumin	5	2.00	—	6.5	28	Water
	Crys. Ovalbumin	5	2.40	—	8.0	38	Water
	Crys. Ovalbumin	5	1.60	—	8.0	30	Water
1-17-23	Fresh egg white	24	—	0.20	—	23	0.8 per cent NaCl
	Fresh egg white	12	—	0.70	—	33	0.8 per cent NaCl
4-25-23	Ovoglobulin (a) + ovomucin	5	—	0.20	>8.0	38	N/500 NaOH
	Ovoglobulin (a) + ovomucin	5	—	0.50	>8.0	41	N/500 NaOH
4-24-23	Ovalbumin (a) + conalbumin + ovomucoid	5	—	0.50	>8.0	33	N/500 NaOH
	Ovalbumin (a) + ovomucoid + conalbumin	5	0.80	—	>8.0	40	N/500 NaOH
	Ovalbumin (a) + ovomucoid + conalbumin	5	—	0.20	>8.0	22	N/500 NaOH
5-24-23	Ovoglobulin (b) + ovomucin	5	1.40	—	>8.0	41	N/500 NaOH
	Ovoglobulin (b) + ovomucin	5	0.90?	—	>8.0	26	N/500 NaOH
	Ovoglobulin (b) + ovomucin	5	1.30	—	>8.0	31	N/500 NaOH
	Ovoglobulin (b) + ovomucin	5	0.70	—	>8.0	42	N/500 NaOH
5-24-23	Ovalbumin (b) + conalbumin + ovomucoid	5	1.30	—	>8.0	33	N/500 NaOH
	Ovalbumin (b) + conalbumin + ovomucoid	5	0.70	—	>8.0	40	N/500 NaOH
	Ovalbumin (b) + conalbumin + ovomucoid	5	1.60	—	>8.0	32	N/500 NaOH
	Ovalbumin (b) + conalbumin + ovomucoid	5	1.20	—	>8.0	22	N/500 NaOH
5-30-23	Ovalbumin (b) + conalbumin + ovomucoid	5	1.20	—	>8.0	22	N/500 NaOH
	Ovalbumin (b) + conalbumin + ovomucoid	5	0.80	—	>8.0	26	N/500 NaOH
6- 8-23	Ovoglobulin (c) + ovomucin	5	—	0.50	>8.0	38	N/500 NaOH
	Ovoglobulin (c) + ovomucin	5	—	0.40	>8.0	40	N/500 NaOH
	Ovoglobulin (c) + ovomucin	5	—	0.40	>8.0	26	N/500 NaOH
6- 8-23	Ovalbumin (c) + conalbumin + ovomucoid	5	0.90?	—	>8.0	42	N/500 NaOH
	Ovalbumin (c) + conalbumin + ovomucoid	5	1.30	—	>8.0	39	N/500 NaOH
	Ovalbumin (c) + conalbumin + ovomucoid	5	1.10	—	>8.0	33	N/500 NaOH

With this question in mind an attempt was made to prepare proteins in such a way as to prevent bacterial contamination during the preparation. Merely a gross separation of the proteins was made and there was no effort to isolate them in a highly purified state at first.

Eggs laid within the previous twenty-four hours were used and the whites were diluted with freshly distilled water to seven times their volume. The ovoglobulin and most of the ovomucin were precipitated and filtered off in this way. From the filtrate the remaining proteins, a mixture of ovalbumin, conalbumin and ovomucoid were precipitated with alcohol. These two fractions of egg proteins when dissolved in NaOH and injected gave no reactions.

The experiment was repeated but this time ovalbumin was obtained from fresh egg white by the usual $(NH_4)_2SO_4$ method except that the entire process was carried out in one day and consequently all the ovalbumin was not obtained as crystals and that part which was obtained was not repurified. The ovoglobulin plus ovomucin fraction was precipitated by dilution as before. With these two fractions of egg proteins, fevers were elicited. A 0.5 per cent $(NH_4)_2SO_4$ solution however, did not produce a febrile reaction.

One more attempt was made to repeat this experiment and the two fractions, an ovoglobulin plus ovomucin and an ovalbumin were prepared as in the previous experiment. Negative reactions were obtained with the globulin fraction but positive with the albumin fraction.

It is evident that more work should be carried out with the egg proteins but the incomplete evidence so far offered is promising and suggestive that some, if not all, of the egg proteins in their purified state will not produce fever.

The same suggestion was applied to a study of edestin from the hempseed. One lot of the protein, consistently gave no febrile reaction in eight experiments on four different days, as seen in table 5. A sample of edestin prepared at the Connecticut Experiment Station gave a marked fever. A third preparation, made in the usual way except that freshly distilled water was used throughout and the entire process was completed in one day, gave fever reactions. The inconstancy of the results with different lots of edestin points to the necessity for further experimentation with this protein, as in the case with the egg proteins, before conclusions can be drawn.

A few experiments were performed with whole blood, serum and hemoglobin. The blood from two dogs, *a* and *b*, was used but the results obtained were not consistent. As can be seen from table 6 the whole blood taken from dog *a* and injected immediately produced no reaction while that from dog *b* produced a fever. The serum injections always were followed by fever except in one case where there was a marked fall in temperature.

The hemoglobin was prepared on the same day that the whole blood was injected and it was tested the following day. The protein was obtained by laking the blood with freshly distilled water and then adding absolute

TABLE 5

DATE OF EXPERIMENT	PROTEIN INJECTED	TEMPERATURE VARIATION +	TEMPERATURE VARIATION −	NUMBER OF ANIMAL	SOLVENT USED	pH
		°C.	°C.			
5–23–22	Edestin A	—	0.50	8	10 per cent NaCl	6.6
	Edestin A	—	0.50	9	10 per cent NaCl	6.7
5–30–22	Edestin A	—	0.80	21	N/500 NaOH	>8.0
	Edestin A	—	0.60	24	N/500 NaOH	>8.0
	Edestin A	—	0.80	30	N/500 NaOH	>8.0
	Edestin B	—	0.30	22	8 per cent NaCl	6.8
	Edestin A	—	0.10	31	8 per cent NaCl	6.8
	Edestin B	—	0.70	32	8 per cent NaCl	6.8
1–29–23	Edestin B	1.60	—	25	N/500 NaOH	>8.0
	Edestin B	0.90	—	28	N/500 NaOH	>8.0
6– 8–23	Edestin C	1.10	—	44	10 per cent NaCl	
	Edestin C	0.60	—	41	10 per cent NaCl	
	Edestin C	0.90	—	30	10 per cent NaCl	

TABLE 6

DATE OF EXPERIMENT	PROTEIN INJECTED	TEMPERATURE VARIATION +	TEMPERATURE VARIATION −	NUMBER OF ANIMAL	AMOUNT INJECTED
		°C.	°C.		cc.
5–28–23	Whole dog blood *a*	—	0.40	36	3
	Serum dog blood *a*	0.80	—	22	4
6– 1–23	Hemoglobin *a*	—	0.70	42	5 (diluted)
	Hemoglobin	—	0.20	40	5 (diluted)
	Hemoglobin	—	0.60	41	5 (diluted)
	Hemoglobin	Died		22	5 (strong)
	Hemoglobin	—	0.60	26	5 (strong)
6– 4–23	Whole dog blood *b*	2.10	—	36	5
	Whole dog blood	0.50	—	31	2
	Serum dog blood *b*	0.90	—	26	3
	Serum dog blood	0.70	—	38	5
	Serum dog blood	—	1.10	32	5
6– 5–23	Hemoglobin *b*	—	0.80	33	5 (diluted)
	Hemoglobin	—	1.00	31	5 (strong)

alcohol. After standing in a freezing mixture, then centrifuging, washing, and dissolving in a small amount of water, the per cent of hemoglobin was determined colorimetrically. By dilute solution is meant one containing

5 mgm. hemoglobin in 5 cc. of solution; by strong, 375 mgm. in 5 cc. solution. The reactions with the dilute solutions were mostly negative, while with the strong solutions one animal died as if from shock, another gave a doubtful reaction, and a third showed a fall in temperature. The one which died, however, had been injected previously with serum from the same dog.

The results, therefore, are too scanty and variable to permit of any deductions except that the few negative reactions obtained here again point to a promise for future efforts.

SUMMARY AND CONCLUSIONS

Of twenty-one proteins, made and preserved in the usual manner, all gave febrile reactions when injected, except the alcohol-soluble ones. Of these latter—six in number—two gave negative results constantly, three gave some positive results, and one gave only positive reactions.

Casein when prepared in the usual manner always produced fever. The amount of casein injected did not seem to be an important factor since 50, 5 and 1 mgm. when injected in the same volume of fluid produced equally intense reactions. Furthermore there was never any evidence of anaphylaxis in the rabbits regardless of how many previous injections or at what intervals they were given.

Extensive processes of purification of casein showed that impurities such as fat, salts and alcohol-soluble products were not the cause of fever, but the few negative results obtained suggested the presence of a pyrogenic contamination in the reactive preparations.

With a knowledge of the cause of the pyrogenic property of some distilled waters, casein was prepared in a manner to prevent bacterial contamination. This preparation gave no fever. The experiment was duplicated. Non-pyrogenic casein, however, may become pyrogenic on standing under non-sterile conditions, again pointing to the bacterial nature of contamination.

Fresh, practically sterile milk, containing only 3000 to 4000 organisms per cubic centimeter, gave fever in doses larger than 1 cc. Therefore, either sufficient bacterial toxic products are formed in vivo in the milk or else there is some other constituent of the milk than fat, salts, and casein which is able to elicit fever in rabbits.

Fresh sterile egg white did not produce a fever. Although ordinary crystallized ovalbumin injections were followed by fever, some suggestive negative results were obtained with egg proteins prepared in a manner to eliminate bacterial contamination. Similarly promising but inconclusive negative results were obtained with certain samples of edestin and hemoglobin. While the results with these three proteins have not as yet

been adequately confirmed, they are presented as promising evidence and merely suggest to the authors that completely satisfactory conditions have not yet been obtained for their preparation as in the case with casein.

These results, together with the numerous negative reactions obtained with the alcohol-soluble proteins, where bacterial contamination would necessarily be checked by the method of preparation, and the knowledge of the cause of water fevers, would seem to indicate that protein per se may after all not be the cause of fever in the so-called "protein fevers."

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THE EXCITATION OF GASTRIC SECRETION BY APPLICATION OF SUBSTANCES TO THE DUODENAL AND JEJUNAL MUCOSA

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Pavlov (1) was the first investigator to observe that the gastric glands could be excited from the intestine. The details of his experiments have not been available. He believed that the secretion following the application of the exciting substance to the intestinal mucosa is reflex in nature, because when such substances were applied per rectum the exciting substance disappeared without gastric secretion being stimulated.

Le Conte (2) reported that gastric secretion could be reflexly excited and inhibited from the intestine. He experimented on dogs with a gastric and a duodenal fistula. He obstructed the intestine above the duodenal fistula, through which he also injected the solutions to be studied. The gastric secretion was collected from the gastric fistula. Peptone caused a definite stimulation of gastric secretion when put into the intestine; meat juice caused a slight secretion; fermented cheese, a slight secretion; casein, milk and Liebig's meat extract were negative. He observed that glucose and saccharose (10 to 25 per cent) caused inhibition of gastric secretion. Le Conte's data demonstrated the efficiency of peptone, but in our opinion his data on the other substances are questionable because of small changes in secretory rate, the lack of sufficient control periods and the type of his animal preparation.

Sokolow (3) and Labasow (4), working on animals with a Pavlov pouch, an "obstructed" stomach—obstructed at the pyloric sphincter,—a duodenal and a gastric fistula communicating with each other by an external rubber tube, observed that food substances (meat, water, extractives of meat, digested egg white) when put into the intestine of such an animal would not have any influence on the secretion of the Pavlov pouch, but when they were retained in the "obstructed" stomach, would cause secretion in the Pavlov pouch.

We have been unable to obtain detailed reports of the work of Sokolow and Labasow and consider, in the light of modern knowledge of gastric secretion, the data presented by Pavlov in his book too incomplete to prove the above claims, because they fail to consider the continuous secre-

tion, report no acid values and the amounts of secretion collected are so small.

Gross (5), working on two animals prepared similar to those of Sokolow with the exception that the obstruction was made at the junction of the fundic and pyloric mucosa, observed that when meat extract was put into the intestine a stimulation of the gastric glands resulted. This apparent contradiction of the findings of Sokolow and Labasow he explained by assuming that the meat extract flowed back into the pyloric antrum and there had its effect. We do not believe that such an assumption is plausible and since Ivy and Whitlow (6) failed to observe stimulation of gastric secretion following the application of meat extracts to the mucosa of the isolated pyloric antrum, we believe that the stimulation observed by Gross was from the intestine.

Krschischkowski (7) and G. Zeliony (8) have made observations on dogs prepared according to the method of Gross. The former investigator observed that Liebig's meat extract, meat, peptone and milk caused stimulation of gastric secretion when put into the intestine. His data have not been available to us. Zeliony observed that Liebig's meat extract and sodium oleate stimulated gastric secretion when put into the intestine. Both of these investigators believed that the stimulation was due to contact of the substances with the pyloric mucosa.

Pointkowski (9) also observed that the introduction of sodium oleate into the intestine excited a flow of gastric juice.

Studzinski (10) reported that neutral fat when put into the intestine inhibits gastric secretion. Sokolov (11) observed that egg yolk, which contains fat, does not stimulate gastric secretion when introduced into the intestine. This investigator also reported that soda solution acts like water when the glands are acting, but has no effect if the glands are inactive. Lönnquist (12) was of the opinion from his work that Na_2CO_3 inhibits gastric secretion when introduced into the intestine.

Chittenden, Mendel and Jackson (13) observed that alcohol introduced into duodenal loops stimulates gastric secretion.

Edkins and Tweedy (14) reported stimulation of gastric secretion when 2 per cent Herzen's meat extract was put into the duodenum of cats studied by their method.

Tomaszewski (15) working chiefly on dogs with a gastric and a duodenal fistula observed that when 5 to 10 per cent peptone (Witte) was put into the intestine, gastric secretion was stimulated. He believed that the stimulation was the result of the procedure of his experiment ("psychic"), because when the peptone was injected over a long period the stimulation was greater and when the vagi were cut the secretion did not occur and the occurrence of the response was irregular. He did not prove, however, that on the days the dogs did not respond, and that in the dog with the

vagi cut, the gastric glands would respond to stimulation. He also observed that 5 and 10 per cent solutions of Liebig's meat extract caused stimulation of gastric secretion when introduced into the intestine even in a dog with both vagi cut, but he explained this result as due to regurgitation of the extract solution into the stomach, although he reports in his data only one instance where such regurgitation actually occurs. The latent period of this stimulation was from 20 to 45 minutes, which is the same as if the extract was given by stomach tube. Meat digested with gastric juice was negative. Soap solution introduced into the duodenum stimulated, but because he observed variations in response, he interpreted this observation as meaning that the chemical property of the soap plays no rôle and that the stimulation was due to a psychic effect. He found water and 5 and 9 per cent NaCl solutions to be negative. Again he failed to prove the gastric glands to be responsive on the day of the experiment.

We are of the opinion that the literature does present positive evidence that gastric secretion can be excited from the intestine, but believe that the data presented and the animal preparations used for experimentation are very unsatisfactory.

METHODS. Because of the unsatisfactory nature of the type of animal preparations used by other investigators for the study of this problem,—they were unphysiological and more or less acute,—we decided to make a preparation that was physiological and one that would live indefinitely, so that we might control our observations more accurately.

Our dogs were prepared by making a Pavlov pouch and Thiry's fistula of the duodenum and jejunum. When the operation was done in two stages, there was no mortality; but when done at one operation, the mortality was about 30 per cent. The Thiry's fistula was made by resecting from 15 to 18 inches of the duodenum and jejunum, the upper transection being made about one inch below the duct of Santorini. The remaining portions of the duodenum and jejunum were approximated by an end-to-end anastomosis. The distal end of the isolated intestinal loop was closed and the proximal end was brought to the exterior through a stab-wound fistula in the abdominal wall. This produced a loop of duodenum and jejunum from 15 to 18 inches long to which substances could be continuously applied and the resulting effect on the secretion of the Pavlov pouch observed.

We have experimented on nine dogs prepared as above, which have lived from one month to nine months in excellent state of health. One dog died of obstruction at the site of the end-to-end anastomosis caused by the formation of an excessive amount of scar tissue and two others died of intestinal obstruction caused by an intra-abdominal hernia, all the intestines herniating above and posterior to the intestinal loop.

In two dogs a Heidenhain pouch was made instead of a Pavlov pouch.

Our experimental procedure consisted of the following steps: *a*, Two one-hour collections of continuous secretion of the Pavlov pouch were made as a control; *b*, application of the substance to the duodenum for 15 to 60 minutes; *c*, two- or three-hour collections of the secretion of the Pavlov pouch were made after the application; *d* and, in the case of no stimulation, a meal was fed to prove the physiological activity of the glands of the Pavlov pouch. We consider this last step obviously very important. It is a point, however, that most investigators on gastric secretion have failed to consider.

The continuous application of a substance to the mucosa of the loop was made possible by a modification of the apparatus described by us in a previous paper, the modification being the connection of a rubber tube 12 inches long, open at the end and perforated 1 inch from the end, to the glass tube connected with the syringe.

The perfusing system, including the intestinal loop and the apparatus, had a capacity of about 100 cc., the loop taking from 40 to 50 cc. The solution was not subjected to a greater pressure than 10 cm. of water, other than at the time when the loop was being filled with the solution.

The contractions of the loop forced the solution back into the syringe or out through the opening of the apparatus, which was always patent. On relaxation the solution passed back from the syringe into the loop. At irregular periods very vigorous contractions occurred which forced all of the solution back into the syringe or out of the perfusing system. Three types of movements were observed, as judged from the passage of the solution back into the syringe or to the outside through the glass tube. One movement, which occurred rhythmically three or four times a minute displacing from 2 to 5 cc. of solution; a second movement, which did not occur rhythmically and was manifested by a gradual displacement of from 10 to 15 cc. of solution, which was interpreted by us as a gradual increase in tone, and a third non-rhythmical, rapid, movement which displaced from 10 to 40 cc. of solution, which we interpreted as being due to a very vigorous peristalsis, sometimes passing toward the closed end of the loop and sometimes toward the open end of the loop, occurred.

We have considered the possibility of psychic factors and of abnormal intra-intestinal pressure having a possible influence on our observations, as will be shown by controls referred to later under our results.

After the period of application all of the solution was removed from the loop, but the loop was not washed. The skin about the fistula was cleaned and, of course, the animals were not permitted to lick themselves.

We should mention that when applications were first made to a loop that had not received applications either defecation or vomiting would frequently occur.

RESULTS: *Hydrochloric acid.* When N/10 HCl is applied to the mucosa of the intestinal loop for from 30 to 60 minutes, a marked secretion of the Pavlov pouch occurs to the same degree as that which is frequently observed following a meal, both in quality and quantity (table 1). Stimulation occurs when N/80 HCl is used, but it is only slight. The latent period is approximately 45 minutes and the augmented flow seldom lasts more than 2 hours. If the gastric glands are in tone, i.e., secreting free acid, the stimulation is more marked than if the glands are not in tone. We did not invariably observe stimulation, but in such cases the glands of the pouch were refractory to a meal. We observed also that when N/10 HCl is applied every day for 4 or 5 days, the mechanism concerned in the stimulation becomes refractory and either no or only a slight secretion occurs. If acid is not applied, then, for several days, stimulation again results on the application of acid. We did not observe this effect if N/20 HCl was used.

In several of our dogs, detailed observations being made on one dog only, we observed that after the first one or two applications of N/10 HCl an enormous secretion of gastric juice from the pouch occurred following a meal. The intestinal loop also secreted large amounts. The secretion of the loop was always augmented following the application of acid. This hypersecretion only occurred when applications were first made to the mucosa of the loop (table 3).

When N/10 HCl is applied to the mucosa of the loop, the solution assumes a brown color in from 15 to 30 minutes and if applied for several days in succession the fluid may return dark brown in color. This does not occur when more dilute solutions are used. This shows that the N/10 HCl has a definite injurious effect on the mucosa.

When a Heidenhain pouch dog is used instead of a Pavlov pouch dog, acid also causes stimulation of gastric secretion when applied to the duodenal mucosa.

We have also made experiments on two men. Two tubes were swallowed and one was allowed to pass into the duodenum. N/20 HCl was then injected into the duodenum and stimulation of gastric secretion resulted.

One gastric fistula dog was used, but so much bile and intestinal contents resulted in most cases that accurate observations could not be made. Stimulation of gastric secretion always occurred, however.

When N/20 HCl and N/20 $NaHCO_3$ were applied alternately to the mucosa of the intestinal loop, no stimulation of gastric secretion occurred except in one experiment in which a definite augmentation of secretion resulted.

Combined acid does not stimulate, as is shown by our results on applying digested meat.

TABLE 1

The effect of the application of substances to the duodenal and jejunal mucosa on gastric secretion

Thiry's fistula and Pavlov pouch dogs

SUBSTANCE APPLIED	NUMBER OF EXPERIMENTS	GASTRIC SECRETION; TYPICAL EXPERIMENT						REMARKS
		Procedure	Time	Amount	Free acidity*	Total acidity**	Pepsin	
			hrs.	*cc.*			*mm.*	
N/10 HCl	20	Control	1	1.0	0	25		Applied 50 minutes
		application	1	1.0	0	15		
			1	9.2	77	97	4.0	
			1	3.5	80	105	3.5	
			1	2.0	40	77		
N/20 HCl	15	Control	1	3.3	52	62	5.9	Applied 50 minutes
		application	1	8.2	85	97	4.5	
			1	6.1	80	90	5.0	
			1	3.7	57	67	6.0	
		Meal	3	20.1	80	105	5.0	Meat
5% soap solution	6	Control	1	1.9	0	12		Applied 45 minutes
		application	½	4.2	50	65		
			½	6.8	90	105		
			½	2.5	60	75		
10% alcohol	8	Control	½	3.5	0	5		Applied 30 minutes
		application	½	4.0	17	37		
			½	9.0	60	75		
			½	5.4	85	105		
			1	1.0	7	35		
10% glycerine	4	Control	1	2.0	0	10		Applied 30 minutes
		application	1	4.9	32	45		
			1	2.6	65	75		
			1	2.0	27	45		
			1	1.5	10	25		
5 % glucose	5	Control	1	1.0	0	12		Applied 30 minutes
		application	½	4.5	25	42		
			½	4.0	42	65		
			½	3.2	37	62		
			½	3.0	15	47		
50% ether	1	Control	½	1.0	0	5		Applied 30 minutes
		application	½	4.0	12	32		
			½	5.5	80	90		
			½	1.5	80	90		

* Acidity expressed in clinical units. Only one control period of continuous secretion is shown; from 2 to 3 hours of continuous secretion was always collected as a control.

Gastric juice. When dog's gastric juice was applied to the intestinal loop, a stimulation of gastric secretion occurred.

Organic acids. N/10 butyric acid causes only a very slight stimulation of gastric secretion when applied to the mucosa of the intestinal loop, whereas N/2.5 causes a definite stimulation.

One per cent acetic, lactic, malic and succinic acids all cause a definite stimulation of gastric secretion, when applied to the duodenal mucosa.

The fact that stronger concentrations of the organic acids are required, we explain as due to the difference in the ionization between these acids and hydrochloric acid.

Alkalis. N/10 Na_2CO_3, N/10 $NaHCO_3$ and 10 per cent $CaCO_3$ and 10 per cent $CaSO_4$ suspensions have been used. Both $CaCO_3$ and $CaSO_4$ were negative. N/10 Na_2CO_3 and N/10 $NaHCO_3$ were both negative and as judged from isolated experiments slightly inhibited the continuous secretion.

We studied on one dog the effect of an application of N/10 Na_2CO_3 and 1 per cent Na_2CO_3 solution to the mucosa of the loop after the animal was given a test meal. We first made four control experiments to find the normal response of the Pavlov pouch to the test meal and then in succeeding experiments we applied the Na_2CO_3 solution to the mucosa of the loop for one hour after giving the meal. We observed that the application of the Na_2CO_3 solution either had no effect or a light, questionable inhibitory effect.

The Na_2CO_3 solution after being perfused was quite "slimy" and "roapy" in consistency, probably due to increased secretion of mucus.

N/10 NaOH and N/10 NH_4OH did not stimulate when applied.

Soap. Five per cent soap solution (Ivory soap) when applied to the mucosa of the intestinal loop caused a definite stimulation of the gastric secretion. The soap solution after being applied became more "slimy" and "roapy" (table 1).

Alcohol and glycerine. Ten per cent and 20 per cent ethyl alcohol caused a marked stimulation of gastric secretion when applied to the intestinal mucosa. Ten per cent glycerine solution was definitely positive (table 1).

Olive oil. Olive oil failed to stimulate when it was applied, which was also the case when the olive oil was emulsified before application.

Olive oil in emulsion with N/20 HCl stimulates, just as it does in the absence of the olive oil.

Glucose, cane sugar and cooked starch. The application of 5 and 10 per cent glucose solution to the duodenal mucosa induced a definite secretion of gastric juice. The stimulation did not occur as regularly and to the degree that occurred when 10 per cent alcohol or N/20 HCl were used. Ten per cent cane sugar solution was applied in three experiments with a

slight positive result in one experiment only. Five per cent cooked starch solution was consistently negative (table 1).

Sodium chloride and magnesium sulphate. Ten per cent NaCl solution caused a definite stimulation in two out of four experiments. Six-tenths and nine-tenths per cent always gave negative results. Ten per cent magnesium sulphate solution in most experiments caused a slight, but definite stimulation of gastric secretion when it was applied.

Water and peptone. The application of tap and distilled water to the duodenum consistently failed to stimulate gastric secretion with the possible exception of one experiment. The very slight increase in the quantity and acidity of the secretion shown was within the variation that frequently is observed in the continuous secretion.

The application of 10 per cent peptone (Witte) in most experiments caused a definite stimulation of gastric secretion (table 2).

Spinach extract and soy bean sauce. Fresh spinach extract when applied to the intestinal loop caused a definite stimulation. The spinach extract was made by autoclaving the spinach for 15 minutes under 2 pounds of pressure. The juice was then expressed through cheese cloth and applied. Two cubic centimeters of the juice injected subcutaneously failed to stimulate secretion in the dogs used in the above experiment (table 2). Soy bean sauce applied undiluted is definitely positive.

Armour's meat extract. Seven per cent and 15 per cent Armour's meat extract was applied to the duodenal mucosa in sixteen experiments and definite stimulation was only observed in three experiments. The particular extract that gave positive results was old, having stood at room temperature, covered but not sealed, for 4 months. But negative results were also obtained with this meat extract as well as with fresh extracts. So stimulation following the application of Armour's meat extract occurs, but is not constant (table 2).

Meat juice. The meat juice was prepared by grinding round steak and then expressing the juice. The application of this juice was consistently negative. However, if this juice after application was placed in an incubator without preservative so that it would putrefy (24 to 48 hours), a definite stimulation of gastric secretion resulted when it was applied to the duodenum (table 2).

We also observed no stimulation, if the meat juice was prepared by adding 100 cc. of water to 250 grams of ground lean meat, boiled for 5 minutes and then expressed.

Extract of smoked highly flavored sausage was also applied without stimulating gastric secretion.

Digested meat. To 250 grams of ground round steak were added 400 cc. of dog's gastric juice, which was allowed to digest under toluol in the incubator for 24 to 48 hours at 37°C. At the end of this time all of the

TABLE 2

The effect of the application of substances to the duodenal and jejunal mucosa on gastric secretion

Thiry's fistula and Pavlov pouch dogs

SUBSTANCE APPLIED	NUMBER OF EXPERIMENTS	GASTRIC SECRETION; TYPICAL EXPERIMENT					REMARKS
		Procedure	Time	Amount	Free acidity	Total acidity	
			hrs.	*cc.*			
Putrefied aqueous infusion of meat	4	Control	½	2.0	0	15	Infusion in incubator 18 hours applied 30 minutes; 37 units combined acid
		application	½	14.0	15	62	
			½	6.0	55	77	
			½	2.8	57	85	
			½	1.5	25	47	
Putrefied digest. Meat combined. Acid neutralized	4	Control	½	2.0	12	27	Applied 30 minutes
		application	½	6.0	30	50	
			½	4.0	50	75	
			½	2.5	37	62	
7% Armour's meat extract	16	Control	1	2.0	0	20	Applied 30 minutes
		application	1	6.4	45	65	
			1	2.5	25	55	
			1	3.0	0	22	
10% peptone (Witte)	4	Control	1	2.0	0	22	Applied 30 minutes
		application	½	3.0	22	42	
			½	4.4	57	75	
Spinach extract	4	Control	1	2.7	5	17	Applied 30 minutes
		application	1	2.6	0	12	
			1	7.5	57	82	
			1	4.1	62	87	
1/1000 histamine	8	Control	½	1.5	0	10	Applied 30 minutes latent period longer than usual
	8	application	½	1.5	0	10	
			½	10.0	60	67	
			½	2.7	60	70	
			½	2.2	12	30	
1/50,000 adrenalin	3	Control	1	3.5	0	17	Applied 30 minutes
		application	½	4.1	20	37	
			½	7.3	95	105	
			½	3.0	92	102	
			½	2.0	80	92	

free acid was combined. When the expressed juice from this mixture was applied, no stimulation occurred.

If the same mixture was allowed to become slightly putrid (24 to 96 hours) and then applied, stimulation occurred. Stimulation occurred also when the combined acid was neutralized by Na_2CO_3. If the meat had a slight putrid smell, it would stimulate (table 2).

Milk, milk whey and casein. The application of fresh raw milk was consistently negative, as was milk whey.

Casein digested for 24 hours with gastric juice was also negative. Only two experiments on each of the substances have been tried up to the present time, which we feel are insufficient.

Ether. We have used ether because it is known to stimulate the flow of pancreatic juice when put into the duodenum. When we applied 50 per cent ether in water to the duodenum, definite stimulation of gastric secretion resulted (table 1).

Barium chloride. Barium chloride solution was used in order to ascertain if motility might be the cause of the stimulation we have been observing. But when 1 per cent barium chloride was applied to the duodenum, no stimulation resulted, although marked motility and blanching of the mucosa, as judged from the rosette of the Thiry's fistula, occurred.

Mustard oil. Mustard oil was used to ascertain if the stimulation might not be due to irritation of the mucosa. Five-tenths per cent musard oil emulsion in water when applied caused no stimulation of gastric secretion.

Histamine. We have used histamine because it is known to be a normal constituent of the contents of the intestine, it stimulates gastric secretion when injected subcutaneously and it occurs in putrefying protein solutions.

When 1/1000 solution of histamine is applied to the duodenal mucosa for 20 or 30 minutes, a marked secretion of gastric juice occurs regularly and without fail. The latent period is from 10 to 30 minutes. Sometimes the greatest amount of gastric juice is secreted during the half-hour of application, and sometimes during the half-hour after application. The augmented secretion lasts from 1 to 2 hours. A dose of atropine, which is known to prevent the gastric glands from secreting to a meal, only slightly inhibits this histamine stimulation (table 2).

Cocaine. When 0.5 per cent cocaine hydrochloride solution is applied only slight stimulation occurs, if any. The response is questionable.

If N/20 HCl solution is applied to the duodenum after 0.5 per cent cocaine hydrochloride has been applied sufficiently to produce local anesthetization, as occurs in other mucous membranes, the usual stimulation of gastric secretion occurs. This is also true when histamine is applied after the cocaine.

Adrenalin, pituitrin and "epinine." When 1/50,000 adenalin solution is applied to the mucosa of the loop, definite stimulation of the gastric secretion results (table 2).

One to ten obstetrical pituitrin solution when applied causes a very ight, if any, stimulation.

"Epinine," 3–4 dihydroxyphenylethylamine, has the "therapeutic effects of adrenaline." When 1/1000 solution of "epinine" is applied to the mucosa, a definite stimulation of gastric secretion occurs.

Amino acids. Tyrosine, glycocol and asparagin have been used up to the present time in our experiments. Only one dog has been used for these experiments.

When 500 mg. of tyrosine were dissolved and partly suspended in 150 cc. of water and applied to the duodenal mucosa, a definite stimulation frequently resulted.

Two per cent glycocol suspension, when applied, does not stimulate.

Seventy-five hundredths per cent asparagin was applied twice in one dog without stimulation occurring.

INCIDENTAL OBSERVATIONS: *Latent period of stimulation.* The latent period of the excitation of gastric secretion from the duodenum varies with the different substances used. When hydrochloric acid was used the latent period varied from 45 to 60 minutes; with glucose it was from 20 to 30 minutes; with peptone, putrefied meat juice, soap and alcohol, from 20 to 30 minutes; and with histamine, from 10 to 30 minutes. The latent period was not accurately determined for the other substances.

It is to be noted that this latent period is approximately equivalent to that of the "chemical" or "secondary" secretion of gastric juice following the ingestion of a meal.

Amount of solution absorbed and neutralized. This was only determined for glucose, HCl and water.

From 25 to 33 per cent of the glucose was absorbed along with 25 to 30 cc. of water when a 5 or 10 per cent solution of glucose was applied for from 30 to 45 minutes to the duodenal mucosa.

From 20 to 40 cc. of water were absorbed from most of the applied solutions in practically every instance.

When N/10 HCl was applied to the duodenum for from 30 to 60 minutes from 15 to 30 cc. of water were absorbed and about 50 per cent of the acid was neutralized and combined, 25 to 30 per cent being neutralized. Chlorine was not absorbed, because the chlorine content of the solution was the same after perfusion as before. This shows that bases pass from the wall of the intestine into the lumen to neutralize the acid and that the intestine itself has the power to neutralize acid chyme.

Stimulation of formation and regurgitation of bile into the stomach. When substances that stimulate the flow of gastric juice were applied to the iso-

TABLE 3

Showing hypersecretion of the Pavlov pouch following application of N/10 HCl to intestinal mucosa 2½ weeks after making the Thiry's fistula

PROCEDURE	TIME (O'CLOCK)	GASTRIC JUICE				INTESTINAL SECRETION	REMARKS
		Amount	Free acidity*	Total acidity*	Pepsin		
		cc.			mm.	cc.	
Feb. 28, 1922, Cont.	10–12	4.0	0	17		3.0	
	12–3	4.0	0	17		5.0	
Meal, 3:00 p.m.	3–5	11.0	70	85		4.0	
March 1, Cont.	1–3	3.0	55	77			
N/10 HCl	3–4	2.6	55	87			Applied to loop 60 minutes
	4–5	7.0	82	95			
	5–6	3.6	62	80			
	6–7	2.8	55	67			
March 2, Cont.	11–2	20.8	112	125			Note marked continued secretion
Cont.	2–3	4.5	90	107	2.5		
N/10 HCl	3–4	3.4	85	100	2.0		Applied 45 minutes
	4–5	13.0	107	120	2.0		
	5–6	4.7	102	117	3.0		
Meal	6–9	30.0	112	122	2.0		
March 3, Cont.	8–9	27.2	127	142			
Cont.	9–12	62.0	125	137		71.0	
Cont.	12–4	26.2	90	102	5.0	65.0	
Cont.	4–5	5.0	70	85	5.5	5.0	
N/20 HCl	5–6	3.3	52	62	5.0		
	6–7	8.2	85	97	4.5	14.5	
	7–8	6.1	80	90	5.0	19.0	
	8–9	3.7	57	67	6.0	13.5	
Meal a.m.	9–12	20.1	80	105	5.0	38.0	
	12–5	53.0	120	125		68.0	
	5–9	65.0	120	130		41.0	
	9–12	30.0	120	127		19.5	
	12–1	5.0	95	107	7.0	12.0	
	1–2	3.2	70	85	7.0	8.0	
N/10 HCl	2–3	3.5	62	80	7.0	8.5	Applied to loop 15 minutes
	3–4	7.5	92	102	5.0	15.0	
	4–5	3.4	70	85	6.5	8.5	

* Acidity expressed in clinical units. On March 7 the dog secretion rate was normal.

lated duodenal and jejunal loop large quantities of bile were regurgitated into the stomach. In one dog which had a gastrotomy and a Thiry's fistula of the duodenum and jejunum, we collected from 10 to 40 cc. of bile mixed with gastric juice in from 10 to 20 minutes. This formation and regurgitation of bile occurred in most instances from 10 to 20 minutes following the application of the excitant to the duodenum, hence it preceded the stimulation of gastric secretion, except in the case of histamine where it occurred frequently simultaneouly with the augmented flow of gastric secretion. This phenonemon occurred when hydrochloric acid, histamine and magnesium sulphate were applied, observations not being made on the other excitants. This phenomenon was observed previously by Ivy (19) when N/20 HCl and 5 per cent sodium bicarbonate were injected into the duodenum of man via a duodenal tube. It was also observed that the regurgitation of intestinal fluids into the stomach does not stimulate gastric secretion in man; when hydrochloric acid was introduced, intestinal secretions regurgitated into the stomach, but gastric secretion was stimulated; when sodium bicarbonate was introduced regurgitation of intestinal secretions into the stomach occurred, but gastric secretion was not stimulated, but inhibited.

So regurgitation of intestinal contents into the stomach does not account for the stimulation of gastric secretion that we have observed.

It is of special interest to note that this phenomenon of regurgitation, occurs even when substances are brought into contact with the mucosa of intestinal loop isolated from its normal position in the gastro-intestinal tract and shows that the mechanism concerned is very probably reflex in nature.

"Gastrin" from acid washings of the intestine. The brown fluid that was formed when N/10 HCl was applied to the intestinal mucosa was saved and extracted by the "Koch 'gastrin' method." When this extract was injected subcutaneously in a Pavlov pouch dog, secretion of gastric juice resulted.

General effect of application of substances to the duodenum. When applications were first made to the intestinal loop, defecation and vomiting, sometimes both, sometimes either one or the other, was observed. This did not occur after several applications were made.

When the loop was over-distended, vomiting would frequently occur. In one instance hiccough, or abortive vomiting, was elicited.

No other general effects than those mentioned were observed.

Effect of distention of the isolated loop with air and water. In order to be certain that the stimulation of gastric secretion was not due to distention of the loop with the solutions that we used, we distended the loop with from 50 to 75 cc. of air. When this was done no stimulation of gastric secretion occurred. The same was true when the loop was distended with water.

The fact that we observed negative effects with other solutions is evidence, also, that the stimulation of gastric secretion we have observed is not due to distention of the intestine.

Psychic and procedure effects. Our dogs were tested for psychic secretion by the usual method with the result that the psychic response never was equal to the stimulation that followed the application of the excitants used. No psychic secretion resulted when we allowed the dogs to smell the putrid solutions we used and many of our excitants were odorless. We do not believe that the procedure per se can account for the stimulation, although we present sufficient evidence of nervous connections between the loop and the central nervous system, because of the negative results with certain solutions such as fresh meat extract and digested meat extract. Also, the fact that stimulation occurred in Heidenhain pouch dogs is presumptive evidence, although not direct, that a psychic factor is not responsible for the stimulation observed.

Concentration of solutions. We can say definitely that "hypotonicity" of solutions is not a factor, which is shown by the negative results when water, 0.6 per cent NaCl solution is used. "Hypertonicity" of the solution may be a factor in the case of some of our excitants, but does not explain the secretion that occurs with fresh meat extract, which is negative until it is allowed to putrefy. It does not explain the stimulation that occurs when histamine, N/20 HCl, 5 per cent glucose, etc., are used.

DISCUSSION. We are of the opinion that the animal prepared with a Pavlov or a Heidenhain pouch and a Thiry's fistula is ideal for determining whether gastric secretion can be excited from the intestine. No complicating factors, such as regurgitation of the solution into the stomach, there having an effect, and regurgitation of intestinal contents into the stomach modifying the gastric secretion, enter in for interpretation. The results of the experiments are clear-cut and in themselves do not require interpretation. The possibility of a psychic factor in influencing the results is, of course, possible, but is no more probable, if application of the solution is properly done and the proper precautions are taken, than that which might occur in any other study on gastric secretion. Our controls on this point, including results on Heidenhain pouch dogs, we believe, show that the stimulation is not due to a psychic effect.

The bearing of our observations on the problem of the "secondary" or "chemical" secretion of gastric juice is evident, as substances known to be present in the intestine after the ingestion of a meal, such as peptone, glucose, organic acids, glycerol, free hydrochloric acid and histamine, we have observed to excite a flow of gastric juice when introduced into a loop of duodenum and jejunum.

If there are "secretagogues" in fresh meat and milk, we are certain that they do not excite gastric secretion from the intestine as these substances have no effect when introduced into the intestine.

Additional evidence supporting the physiological significance of our observations is the striking coincidence between the time of the latent period of the intestinal excitants and the time of the latent period of the "secondary" or "chemical" secretion of gastric juice following the ingestion of a meal.

The fact that hypersecretion of gastric juice occurs following a meal (table 3), when acid is first applied to the intestinal loop after the operation, the mucosa of the loop having not come into contact with acid for 2 or 3 weeks, shows that the mechanism concerned in the excitation is especially irritable and that after application of the acid maintains the gastric glands in a state of tone, even after the immediate stimulation of the flow of gastric

TABLE 4

Effect of application of a mixture of known excitants to duodenal mucosa on gastric secretion

PROCEDURE	TIME (O'CLOCK)	GASTRIC SECRETION				REMARKS
		Amount	Free acidity	Total acidity	Pepsin	
		cc.			*mm.*	
Continuous	9–10	2.0	0	5		Mucus
	10–11	2.0	0	5	1.5	
	11–12	1.0	0	5		Latent period 20 minutes
Application of mixture	12–12:30	4.4	42	62		
	12:30–1	7.9	100	115	3.5	
	1–1:30	4.1	97	115	1.5	
	1:30–2	2.0	77	97	1.0	
	2–3	1.5	5	37		
Meal of meat	3–4	9.0	60	80	1.5	
	4–5	12.0	75	95	1.0	

juice disappears, so that food ingested causes a marked and prolonged secretion. This we believe may explain, in part, the hypersecretion that is known to occur in dogs on feeding after a prolonged fast (16), because in such a condition the duodenum has been free from acid, although not complete, and food contact for a long period of time and hence the duodenum and jejunum is in a comparable condition to that of a Thiry's fistula of the duodenum and jejunum. Another significant observation is that successive applications of N/10 HCl to the duodenum and jejunum fatigues or exhausts, or at least renders refractory, the mechanism concerned in the excitation. A similar phenomenon has been observed in the case of acid stimulation of pancreatic secretion as observed by Javois and Ivy (17) in both acute and chronic pancreatic fistula dogs and by Grogan

and Luckhardt (18) in their work. This observation may explain the fact that following the ingestion of a meal the rate of secretion of the digestive juices returns to the normal rate of secretion of the continuous secretion. In other words, this fatigue or exhaustion of the intestinal mechanism for exciting the secretion of the digestive juices is an additional factor, another factor being possibly the emptying of the stomach and duodenum, that possibly causes the decline or ebb in the rate of secretion that occurs from 5 to 7 hours after the ingestion of a meal. That this fatigue or exhaustion of the intestinal mechanism is not the prime factor concerned in the decline of secretion is shown by the fact that in some animals, the herbivora, the digestive secretion is continuous and that a dog secretes longer to a large meal than to a small one.

We hesitate to speculate on the mechanism concerned in the excitation of gastric secretion from the upper part of the intestine, as we have not yet done a sufficient number of experiments to warrant such speculation. However, at this time we feel certain that it is not due to the absorption of water, the distention or motility of the intestine, or irritation of the mucosa. In the case of acid stimulation, the length of the latent period, from 45 to 60 minutes, shows that the mechanism is different, at least in part, from the mechanism concerned in the acid stimulation of pancreatic secretion, in which the latent period is from 7 to 10 minutes. Several mechanisms are possible. For example, the stimulation of secretion may be due to a local secretory reflex from the intestinal mucosa to the gastric mucosa, as Pavlov has suggested, or to a long reflex to the higher centers, having either a secretory or a vasomotor effect, or to the production of a "secretin" in the intestinal mucosa or to the presence of "secretagogues" in the substance applied, or to a "nausea-reflex" that might lead to increased secretion.

Although our observations seem to be physiologically significant, we are skeptical, which attitude of mind will be maintained until definite results are obtained on the mechanism concerned n the stimulation.

CONCLUSIONS

1. Gastric secretion can be excited by the introduction of certain substances into the intestine in dogs prepared with a Pavlov or a Heidenhain pouch and a Thiry's fistula of the duodenum.

2. The most active and constant excitants when applied to the duodenal and jejunal mucosa are as follows: N/10–N/20 HCl, 10 per cent ethyl alcohol, 5 per cent soap solution, 10 per cent glycerine solution in water, putrefied fresh meat infusion, fresh spinach extract, 1/1000 histamine solution, 1/50,000 epinephrin solution and 10 per cent Witte's peptone.

3. Other excitants are reported that have a mild and more irregular stimulating action.

4. N/10 HCl and N/10 Na_2CO_3 applied alternately to the intestinal mucosa results in a definite stimulation of gastric secretion.

5. "Gastrin" is present in the acid washings of the intestinal mucosa and successive applications of acid to the intestinal mucosa cause it to become refractory.

6. Increased formation and regurgitation of bile into the stomach occur when acid, histamine and magnesium sulphate are applied to the intestinal mucosa.

7. The latent period of stimulation varies from 10 to 30 minutes in the case of histamine and from 45 to 60 minutes in the case of hydrochloric acid.

8. Free H-ions are necessary for acid stimulation.

9. The intestine possesses the power of neutralizing acid solutions.

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STUDIES ON THE PHYSIOLOGY OF SLEEP

II. Attempts to Demonstrate Functional Changes in the Nervous System During Experimental Insomnia

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Deprivation of sleep, however produced, results in a group of varying subjective symptoms and a feeling of decreased efficiency in the performance of daily tasks. In a previous paper (1) by one of us it was reported that no variation from the normal could be detected in a large number of functions of the human organism during sleeplessness, and that certain changes could be explained by greater muscular relaxation of the sleepy subject. The purpose of this study was to determine if any changes occur in the activity of the higher and lower centers of the nervous system as a result of prolonged deprivation of sleep. The work of Patrick and Gilbert (2), of Aschaffenburg (3) and Roemer (4) all points to the deterioration of some functions, but insufficient data of normal daily variations in these functions before and after the tests are given to render their conclusions above question. Smith (5), on the contrary, reports the effect of several short sleepless periods on one subject to be increased efficiency immediately following the period of insomnia, succeeded by a few days of gradually decreasing efficiency. Recently Robinson and Herrmann (6) concluded from well-controlled experiments that the results of the tests performed "were not affected by insomnia in any marked or consistent manner." The data obtained by them during the sleepless period fall within the daily variations during the control periods and, in the main, their results were negative.

In our work we followed the method employed by Robinson and Herrmann. Our study has extended over nearly one year with very long control periods between insomnia tests. The daily test occupied about two hours, and for this reason only one subject was employed. He underwent six sleepless periods, three of 60 hours, two of 90 hours, and two of 114 hours. Two of these insomnia tests were made in the fall, two in the winter, one in the spring, and one in the summer. The various determinations were made by the same experimenter and under the same conditions throughout the entire series of experiments. The tests were made once

daily at the same hour, both during the control period and during insomnia, and with each new experiment a new hour was set. On some occasions the tests were made twice each day, in the morning and in the evening. The subject was a male student, 28 years of age of medium height and weight, and in good health. He is a good sleeper normally, and during the control periods adhered to a strict schedule of hours of sleep, meals and work. During the periods of insomnia he ate and worked as usual, but could keep himself awake only by continued activity. An assistant was constantly with him to prevent his falling asleep.

METHODS. In the selection of the tests we wished to include only those which in the hands of other workers had been found capable of demonstrating the effect of fatigue and of drugs, and which were found suitable for long-repeated use and quantitative treatment. The tests chosen fall into two classes: those in which effort can in no direct way be a factor, and those in which it may be one of the factors. The first group consists of the knee jerk, pupillary reflex, determination of the sensory threshold for faradic stimuli, and a test for steadiness. The vascular skin reflex described by Marey, Mueller and others, and used as a quantitative test by Ryan, proved unsatisfactory in our hands. In the second group we include reaction time, naming of opposites, color naming, cancellation and mental arithmetic. We took some records of the frequency of the inversion of the image of a cube or other solid object, when a simple drawing of it was fixated, but did not succeed in standardizing the conditions of the test and therefore abandoned it. Determination of the threshold for auditory stimuli was likewise found impracticable in our laboratory. In all of the tests finally used, except mental arithmetic in which improvement continued throughout the year we were able to reach satisfactory practice levels during the control periods.

Knee-jerk. The subject was seated in a comfortable semi-reclining chair, his leg hanging free from the knee, at right angles to the thigh. A fine non-elastic wire attached to the heel by means of a stirrup was passed over a pulley to a writing point registering on a slowly moving kymograph. This simple time-honored method in which the extent of the kick as measured by the movement of the heel is proportional to the height of the excursion of the writing point proved reliable and accurate enough for comparing the performance of the same individual on different days or under different conditions. The stimulating device consisted of a spring hammer in which the strength of the blow depended upon the degree of compression of the driving spring and could be changed at will, while the extent of excursion of the hammer and the consequent depression of the tendon remained the same, thus rendering the mechanical component of the knee jerk a constant. The various screws and hinges made possible very accurate adjustments, so that the hammer could be made to strike the

tendon perpendicularly and always at the same point. The hammer was released electrically by means of a suitable key, which could be operated at a distance. A sketch and description of this device are given in figure 1.

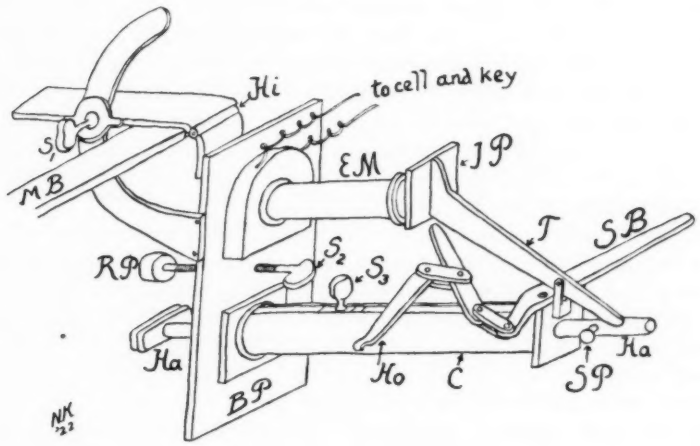

Fig. 1. A sketch of the apparatus used to stimulate the tendon in eliciting a knee jerk. *MB* is the main bar which connects the device with an adjustment-stand or with an adjusting arrangement on the chair on which the subject is seated. By means of adjustment screws not shown on the figure the entire apparatus may be moved up and down, forward and backward, and from side to side. The brass plate *BP* to which the various parts of the apparatus are attached is joined to the main bar by the hinge *Hi* and is generally kept in a vertical position, but can be fixed at any desired angle with the aid of the screw S_1. This makes it possible to use the apparatus with the leg in any position in reference to the thigh. To the plate *BP* is attached a hollow brass cylinder *C* carrying a strong steel spring (not shown) fastened to the stimulating hammer *Ha*. The degree of compression of the spring, and therefore the force with which the hammer is driven, is determined by fastening the end of the spring to the hammer at any point along the cylinder by means of the screw S_3. In setting the apparatus the hook *Ho* is placed in a groove near S_3, and the movable bar pulled toward the stationary bar *SB* until the end of the brass trigger *T* can be fitted into a notch at the end of the hammer. *EM* is an electromagnet connected to a cell and a key at some distance from the subject, and when the circuit is closed the electromagnet attracts the iron plate *IP* at the end of the trigger, turning the latter and so releasing the spring hammer. The side piece *SP* limits the excursion of the hammer, and makes the mechanical displacement of the tendon constant irrespective of the force of the blow. By means of the rubber pad *RP* operated by the screw S_2 the distance from the cross-bar at the end of the hammer to the tendon may be kept constant or varied at will. The apparatus designed by one of us for use in her studies of individual reflex variability now in progress was made by Mr. August Johnson of our laboratory and was found to give very good results.

The first stimulus was given after the subject had been reclining in the chair for 10 minutes, relaxed, silent, and with eyes closed. We obtained consistent results when we averaged ten successive knee jerks elicited at irregular intervals of 15 to 30 seconds each. Our preliminary work showed that if less than 15 seconds elapsed between blows, the second response was markedly affected by the first; in some subjects it was increased, in others decreased. It was also observed incidentally that the extent of the knee jerk was, up to a certain point, directly proportional to the strength of the stimulus and then decreased as the strength of the stimulus increased—because of the stimulation perhaps of the antagonistic flexors as well as the extensors. The optimum stimulus was determined for our subject and thereafter was used throughout the experiment.

Pupillary reflex. The reflex of the pupil to light and accommodation was determined by placing in front of the subject an opaque box containing a powerful electric light and equipped with suitable chin and forehead rests. In the front wall of the box was inserted a large disc of frosted glass which could be covered by a sliding paper screen. When this only slightly translucent screen was in place, enough light would diffuse through to illuminate faintly the face of the subject. When the screen was removed the bright light fell on the pupils of the subject. The observed eye was at a constant distance, about 4 inches from the source of light. The observer looked at the pupil through a telescope from a distance of approximately 8 feet. This telescope contained a fine cross section scale which projected on the image of the pupil gave a very accurate indication of its size. After one minute in which the eye became adapted to the feeble light and the pupil no longer changed in size, a reading was taken, and the screen removed. Ten seconds were given for adaptation to light, and a second reading taken. Up to this point the subject was maintaining a relaxed accommodation. Then at a given signal the subject would accommodate for the bright disc of glass and a reading was taken 2 seconds later. This procedure was repeated three times and the average for the day obtained.

Sensory threshold for faradic stimuli. The method and technique of Martin were followed, and for a detailed description the reader is referred to his original work (7). We assumed that the tissue resistance was the same throughout the experiment.

Steadiness. As there has been no test for steadiness described in which the technique and scoring are wholly satisfactory, we used a method suggested by some unpublished work of Dr. A. I. Gates of Columbia University. A special instrument was devised consisting of a cylinder fastened upright to a head band. The upper end of the cylinder was stoppered with a cork, and through a channel in the cork was passed a wire fastened to a spring located within the cylinder (fig. 2). When this instrument had been firmly adjusted to the forehead of the subject, a horizontal metal

plate covered with smoked paper was lowered on a standard until the paper just touched the end of the vertical wire. The easy compressibility of the spring within the cylinder insured continuous contact of the writing point and the smoked surface and free play over the latter. Still more delicate adjustment was obtained later by attaching the paper loosely to the plate. The subject was told to "stand still" with the eyes closed, as in the Romberg test, and a tracing of the writing point was taken for 2 minutes. As the instrument was perfectly noiseless, he was unable to tell the extent of the excursion of the writing point, and therefore made no especial effort to decrease the magnitude of the oscillations of his body on the days that these were greatest. The records obtained by this method lend themselves only roughly to numerical treatment, but their interpretation is not difficult.

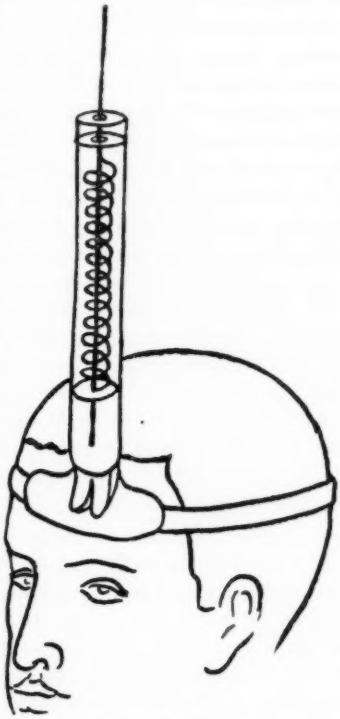

Fig. 2. A sketch of the device used in the steadiness test. The apparatus was strapped to the head of the subject, and the wire allowed to record the swinging of the body by lowering a horizontal metal plate covered with smoked paper until the paper touched the tip of the wire. The fine spring which carried the wire insured continuous contact between the wire and the smoked surface, with the minimum of friction. The recording was entirely inaudible.

Reaction time. For the determination of the reaction time the Johns Hopkins chronoscope described by Dunlap (8), (9) was employed. The instrument we used was made by the Stoelting Company for the University of Chicago, and was provided with a pneumatic key.[1] The reaction time was read off in sigmas. We took auditory and visual reactions. The auditory stimulus was of the "suspended" type, i.e., a telegraph apparatus was clicked at intervals of from 1 to 10 seconds after the signal "ready" was given. Visual reaction was of the choice or discrimination type, the subject being instructed to react to yellow or green light only, and the color of light changed at will by the experimenter. The interval of time between

[1] We are greatly indebted to Professor Robinson of the Department of Psychology for the opportunity to use this delicate and accurate chronoscope.

the signal "ready" and the stimulus in this case was the usual one of one second. Forty auditory time tests were made at each sitting, and thirty-two visual (twenty correct stimuli and twelve incorrect ones, arranged in chance order).

Naming of opposites. We prepared a list of fifty words, such as was used by Bonser, Hollingworth and others. The same words were printed on six cards, in different order on each card. The subject had to look at the card and read off the *opposite* of each word as rapidly as possible, the total length of time it took him to name the fifty opposites being determined by means of a stop watch. Thus on looking at the words "drunk, sacred, stale, hostile, noisy," he would read off loudly "sober, profane, fresh, friendly, quiet." Each day a different card was used, and it was therefore impossible for him to memorize the order of the opposites.

Color naming. On a square piece of cardboard were pasted, in chance order, 100 little squares of colored paper, 1 cm. square and 1 cm. apart, red, yellow, brown, black, gray, violet, green, blue, pink and orange—ten of each color. A different arrangement of colors was obtained by rotating the card successively through an angle of 90 degrees. In our first series we followed exactly the method described by Hollingworth (10). The card was placed face down before the subject and turned over at a given signal. At a second signal the colors were named as rapidly as possible in reading sequence. The experimenter called the subject's attention to every mistake made, and had him correct it before proceeding. The score was the total time necessary to name the 100 colors correctly. In a second series of experiments, after the 100 colors of one card had been named, another card, with the colors in a different order, was immediately placed before the subject, and in this manner the number of colors correctly named in 15 minutes determined. A third series of experiments consisted of a daily record of the time necessary to name 1200 colors (12 cards) without correction of errors. The latter were observed by the experimenter on a duplicate card, and recorded both as to the errors that the subject himself corrected and as to those he left uncorrected. Both this test and the one of the naming of opposites have been found by Hollingworth to show diurnal variation, influence of fatigue and the effect of caffeine.

Cancellation. Printed lists were prepared on which the digits 2 to 9 inclusive appeared each 50 times, making 400 figures in all. They were arranged in rows of 12 and 13 alternately in a chance order. We followed the pattern used by Franz, but extended it in length (11). The subject was told to go through the entire list as rapidly as possible, crossing out a certain one of the digits, say, all the 4's. On a second card he was instructed to cross out two digits, on a third three digits. Different digits or combinations of digits were designated by the experimenter each day, and the time

it took the subject to go through each of the three cards as well as the number of errors recorded.

Mental arithmetic. Two numbers of two digits each were written on a card which was given face down to the subject. At a given signal he turned it over and multiplied one number by the other, mentally, the score being the time it took him to obtain the correct answer. As the subject kept on improving with practice, the test was made harder by using one number of two and another of three digits, and finally two numbers of three digits each. As stated above, we never reached a practice level, the time curve going down continuously, but we hoped to detect a break in the curve in case a lowered ability to multiply mentally should develop during insomnia.

RESULTS. In a general way the various functions were little affected by the loss of sleep. The amount of numerical data we collected during the past year is enormous, and we spent a good deal of time in tabulating and plotting our results. Were not the control periods before and after the insomnia tests of such long duration, the interpretation of our results would have been different. As it is, the values obtained are well within the limits of the diurnal curves, and those who have read the paper by Robinson and Herrmann (6), who obtained similar results, will understand why we refrain from giving tables and curves in this paper. Some functions studied showed, however, very definite changes during sleeplessness, and those we shall report more in detail.

The extent of the knee jerk varied from day to day in our control periods, but only within very definite limits. No change whatsoever could be observed in the knee jerk as a result of sleeplessness. Toward spring the knee jerk disappeared altogether, and could be elicited only by Jendrassik's method of augmentation. At this time it was absent also in insomnia, and when brought out by reinforcement, it did not differ in extent from the knee jerk obtained normally by this method. Incidentally we found that squeezing the hands together tightly or any such powerful outpouring of motor impulses to the skeletal musculature was not usually necessary to augment the knee jerk or to elicit it when absent. Ordinary speaking, as in answering a question, proved an efficient milder method of augmentation. It occurred to us that this method could be of use clinically when an ordinary stimulus fails to elicit a knee jerk. Another interesting observation was the complete disappearance of the knee jerk, if the subject happened to fall asleep during the test. As stated above, he was required to lean back in the chair, silent, with eyes closed, for 10 minutes before the knee jerk was taken. During the periods of enforced insomnia he would often fall asleep under these conditions, and if this occurred no knee jerk was obtained. If, however, the experimenter woke him before releasing the hammer of the stimulating device, a normal knee jerk was obtained (fig. 3). If left undisturbed, the subject, under these

conditions of complete muscular relaxation, would immediately fall asleep again, and this test could be repeated any number of times. It confirms the observation made long ago by Lombard (12) under somewhat different conditions and seems to establish the fact that the knee jerk is lost during sleep.

In measuring the size of the pupil we found that it becomes smaller as a result of sleeplessness. In one experiment the average size of the pupil during the control periods preceding and following the insomnia period was (as measured by the divisions on the telescope scale) 12.16 under faint

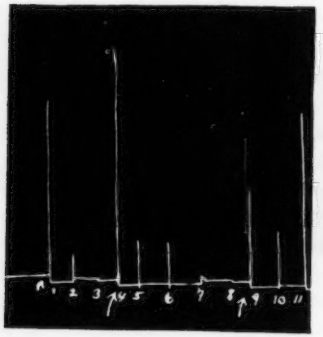

Fig. 3.

A

B

Fig. 4.

Fig. 3. The knee jerk record obtained during a sleepless period. The subject fell asleep before any knee jerk was obtained. The three arrows indicate that the subject was awakened before the stimulus was applied. After he was awakened for the for the third time, he was told to keep his eyes open for the rest of the test. Intervals between successive stimulations, about 30 seconds.

Fig. 4. The record obtained with the device shown in figure 2. *A* is a typical record obtained on March 23, 1923, just before the beginning of a sleepless period. *B* is a record obtained with the same apparatus on March 25, 1923, after two sleepless nights. Both records were taken at 10 a.m. Some records obtained during sleepless periods were twice as large as that shown in *B*.

illumination and 4.50 in bright light; during the period of sleeplessness (average of four daily observations), the corresponding figures for the size of the pupil were 10.55 and 4.68. The change in size or narrowing of the pupil due to bright light was 7.66 normally and 5.87 during insomnia. The narrowing of the pupil as a result of accommodation was negligible under any circumstances, probably because the very bright light caused a maximal contraction of the pupil. Indeed, sometimes a slight dilatation of the pupil could be noticed as a result of accommodation. In another experiment, with the light somewhat stronger, the pupil changed in response to light from 8.18 to 5.63 during the normal control period, and from 7.00 to 4.66 during insomnia.

In the sensory threshold for faradic stimulation, in the reaction time to auditory and visual stimuli, in the naming of opposites, in the cancellation, in the mental arithmetic tests, in none of these could changes be detected during sleeplessness that did not fall within the limits of daily variations during the control periods. During some insomnia periods we found, indeed, changes in these, but could not confirm them in subsequent tests. Insofar as color naming is concerned, we found no changes as a result of sleeplessness during the first series of experiments, when we used only one card, but in later experiments, when several hundred colors had to be named, and the attention had to be fixed on the color naming for as long as 15 minutes, we did detect an effect of insomnia. This was especially noticeable during the third series of tests, in which the subject, if he made an error, was not stopped for its correction. Thus during the control period of one experiment it took him 749.4 seconds on the average to name 1200 colors, with a daily average of 9 errors corrected and 4.2 unnoticed and therefore uncorrected. During insomnia he spent on the average 773.3 seconds in naming 1200 colors, and the average number of corrected mistakes rose to 12.7, and of uncorrected to 5.7. In another insomnia period of longer duration the change was more marked, as the subject spent on the average 792.5 seconds in naming 1200 colors as compared with 742.2 during the control period.

In the steadiness test the changes as a result of sleeplessness were very marked. In figures 4 are shown some of the records made by the instrument attached to the subject's forehead. As the subject had his eyes closed and the writing device was noiseless, he had no idea how well or how poorly he was doing, and was very often surprised upon seeing the record he made. Occasionally the oscillations of the subject's body were rather wide under the normal control conditions but they were invariably so during insomnia.

Discussion. The only purely skeletal reflex function we studied, the knee jerk, showed no change as a result of sleeplessness. Although the method of recording in which the leg itself is used as a lever is admittedly crude, we are satisfied that had there been a change in the extent of the knee jerk we would have detected it. Thus in other experiments (to be reported soon) on the effects of certain drugs and of prolonged fasting on the knee jerk we used the same stimulating and recording devices, and had no difficulty in detecting marked changes in the reflex. Yet the onset of sleep abolishes the knee jerk altogether, and awakening restores it immediately. This is especially significant, because the position of the knee with reference to the spring hammer and the strength of the stimulus is the same in all of these cases. It would seem that as long as the individual is awake, the reflex centers, as judged by the center for the knee jerk, function with undiminished intensity, even though the period of wakeful-

ness is prolonged to several times its normal length, which is 16 hours. It is not necessary to enter here into a discussion whether the knee jerk is a true reflex, but it is generally accepted that its magnitude and even its presence depends upon the tonicity of the muscles involved. Its disappearance with the onset of sleep would indicate a coincident lowering of the tonus in the skeletal muscles.

The visceral reflex we studied, the contraction of the pupil in response to bright light, also shows persistence with practically undiminished intensity during sleeplessness. Unlike the knee jerk it can be elicited during sleep, even in deep sleep that follows a period of prolonged insomnia, although the response is more slugglish than during wakefulness (1). The size of the pupil, however, with dim light is considerably smaller than normal. There are so many elements involved in the control of the size of the pupil that it is difficult to interpret this finding. It may be due only indirectly to sleeplessness. As the eyes are kept open throughout the entire sleepless period, it may be that the continuous action of light on the retina is responsible for this narrowing of the pupil. We are not warranted in assuming that sleeplessness as such is the direct cause of this narrowing of the pupil.

The various tests involving mental as well as muscular activity of comparatively short duration gave negative results, and in this respect we can fully confirm the findings of Robinson and Herrmann. These investigators caution us against the asumption that the effort in the performance of daily tests, although theoretically always maximal, is practically so. In the long lasting control periods after the novelty of the experience has worn off and the practice level established, the subject takes the daily tests like any other routine task, and is not liable to put the maximal effort into its performance. During the sleepless period, however, the subject is spurred to do his utmost by the desire to show an undiminished capacity for mental and muscular activity. The results obtained with the color naming tests illustrate this possibility. In the first series of experiments where the subject had to name only 100 colors, which he learned to do in about one minute, no deviation from the normal could be observed during sleeplessness. When, however, he had to name 1200 or more colors in succession and had to keep his attention unflagging for 700 or 800 seconds, we found a marked qualitative and quantitative change as a result of sleeplessness. In other words, the effect of increased effort disappeared when the test became one of endurance.

In the steadiness test we obtained unmistakable evidence of the deteriorating effect of the lack of sleep upon the ability to maintain one's equilibrium. It was our impression that effort played no part in this test, and perhaps that accounts for the positive results we obtained. How far the unsteadiness manifested may be explained by simple muscular fatigue

we are unprepared to state at the present time. More work of a comparative nature will be necessary to elucidate this point. As indicated in a previous paper (1), the tests we performed were not strictly insomnia tests. In order to keep awake the subject had to move about practically all of the time and some of the results might be due to fatigue of the neuromuscular mechanism that was made to function uninterruptedly. As the period of insomnia progressed, a tendency toward muscular relaxation became evident and it was increasingly more difficult to keep the musculature in tonus. This alone might account for the progressive inability to preserve equilibrium. It should be remembered, however, that from the practical standpoint sleeplessness always involves a certain amount of muscular activity which invariably accompanies the state of wakefulness, is indeed one of its most characteristic features.

SUMMARY

1. Experiments were performed to detect any functional changes in the nervous system as a result of experimental insomnia in man, the periods of sleeplessness varying from 60 to 114 hours, and separated by long control periods.

2. The knee jerk is not affected by insomnia, but promptly disappears with the onset of sleep.

3. The pupillary reflex persists practically unchanged during insomnia, but in dim lights of standard intensity, the diameter of the pupil is much smaller than normal.

4. The sensory threshold for faradic stimuli, the ability to react to auditory and visual stimuli, to name opposites, to multiply mentally,—all these show no change in insomnia, confirming the observations of Robinson and Herrmann, who used similar tests.

5. The ability to name colors was not impaired during insomnia, if the number of colors was small (100), but when the subject had to name 1200 colors in succession, he spent more time and made more errors than during the control periods, thus showing inability to sustain attention under these conditions.

6. The power to maintain one's equilibriun, applying the graphic method to Romberg's test, showed marked deterioration during insomnia, but this was possibly due to concomitant muscular fatigue.

We wish to thank Dr. A. J. Carlson for his aid in the selection of the tests and the interpretation of the results.

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THE ANURIA FOLLOWING TEMPORARY ANEMIA OF THE KIDNEYS

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The cessation of urine flow during the clamping of the renal vein, and the variable periods of anuria following temporary occlusion and release of the renal artery or the renal vein have played a considerable part in the discussions of urine formation, as also of glomerular filtration. The original observation that clamping the renal artery for a few minutes induces a temporary complete anemia with a gradual return to the normal rate of urine flow or restoring the renal circulation appears to have been made by Hermann, but with no detailed report of the work. The literature is reviewed by Marshall and Crane (1). The general acceptance by physiologists of temporary renal anemia as inducing anuria outlasting the period of anemia by many minutes, if not hours, is probably based on their own observations rather than on the work of Hermann. Apart from research on the kidney, teaching experiments on various aspects of renal function are given in most physiological laboratories. In such experiments in our laboratory during the last twenty years we have noted *a*, the frequent induction of reflex anuria (renal vasoconstriction?) from handling the pelvic viscera, as well as the anuria induced by temporary occlusion of the renal artery under conditions excluding reflexes into the kidney. Marshall and Crane, in recent reports, find that anemia, even of 20 minutes duration, does not cause anuria, if reflex vasoconstriction in the kidney is avoided. Temporary renal anemia changes the character of urine, but this altered urine starts to flow from the ureter within a minute of the release of the clamped artery. Marshall and Crane are inclined to ascribe the anuria reported by previous workers as due to reflex vasoconstriction in the kidneys.

The present work was undertaken with the view of determining whether the belief that anemia of the kidney of short duration induces prolonged anuria is an error, and, if not an error, whether an explanation of the anuria can be found in the vascular condition of the kidney induced by the anemia. On this last point there appear to be few or no recorded observations. Barcroft (2) has published two experiments indicating marked reduction in the blood flow through the kidney after 10 minutes and 18

minutes clamping of the renal artery. According to Barcroft's experiments, such renal anemia stops the diuresis induced by caffein (true secretion?), while it does not stop a purely saline diuresis (glomerular filtration?). It should be noted in this connection that in all of the experiments reported by Marshall and Crane diuresis was induced by intravenous injection of varying quantities of hypertonic (10 per cent) sodium chloride solution and other crystalloids.

EXPERIMENTAL METHODS. Dogs under veronal anesthesia were used in all our experiments. Occasionally it was found expedient to administer a little ether to induce greater relaxation of the abdominal muscles. The dogs were covered with cotton and towels so as to avoid subnormal body temperature.

TABLE 1

Dogs. Veronal anesthesia. Urine flow recorded in drops from cannulated ureters. Anuria following clamping and release of renal artery or renal vein. Continuous renal activity aided by water by mouth (stomach tube) or periodic intravenous injection of small quantities of Ringer's solution

NUMBER OF EXPERIMENTS	PERIOD OF RENAL ARTERY OCCLUSION	PERIOD OF ANURIA AFTER RESTORING CIRCULATION		
		Minimum	Maximum	Average
	minutes	*minutes*	*minutes*	*minutes*
4	1	10	10	10
5	2	10	25	12
18	3	5	35	11
2	4	5	20	12
13	5	5	110	25*
10	6	5	50	23
14	7	5	20	15
6	8	5	20	15
13	15	10	150	50**
9	20	5	80	50
8†	5	5	25	14
7†	10	15	30	20
4†	15	15	30	20

* Excluding experiment with the latent period of 110 minutes.

** Excluding experiment with latent period of 150 minutes.

† Occlusion of the renal vein.

As a routine procedure the dogs were given 150 to 200 cc. of water by stomach tube before being anesthetized so as to insure a good urine flow. In cases where the urine flow was still slow or irregular, intravenous injections of small quantities of Ringer's solution at fixed intervals were resorted to. In no case did we use hypertonic solutions.

As a rule both kidneys were used in the experiments. They were isolated from the ventral side, and cannulae inserted in the ureters, close to the

bladder. In every case the renal plexus of nerves was separated from the renal artery so as not to be included in clamping of the renal artery or the renal vein. In some animals the kidneys were denervated some weeks before the crucial experiment. In other cases the renal nerves were sectioned just before the experiment began, or during the experiment. A number of experiments were run with the renal nerves isolated from the renal vessels but left in connection with the central nervous system.

The rate of the urine secretion was measured by the drop method. The renal anemia was produced by placing a rubber-covered bull-dog clamp on the isolated artery or vein.

RESULTS. 1. *The anuria following temporary renal anemia.* Our results on this point are summarized in table 1. The following points may be added to the data given in the table:

1. In no case where the anemia of the kidneys was complete did the urine flow return immediately on releasing the clamped vessels. The duration of the anuria is variable, but roughly proportional to the length of the anemia.

2. In three cases of occlusion of the renal artery (3 minutes, 15 minutes and 20 minutes) and in one case of occlusion of the renal vein (10 minutes) the anuria lasted for the duration of the experiment (2 to 3 hours).

3. The urine may continue to flow with gradually decreasing rate for a few minutes after occlusion of the renal vein or artery.

4. The rate of the urine flow after the occlusion anuria is usually less than before the anemia is induced. The rate increases gradually, and may eventually equal or exceed the control rate, but it usually remains subnormal.

5. A second and a third period of anemia of the same kidney usually cause greater impairment of urine production than does the initial occlusion.

6. In two experiments the period of anemic anuria was distinctly longer in the kidney with the renal nerves intact than in the other kidney which was denervated. But these differences fell inside the limits of individual variations.

2. The dilution of the urine caused by temporary anemia of the kidney. According to Barcroft, the true secretory functions of the kidney are depressed or temporarily abolished by temporary renal anemia. It has been known for a long time that even a temporary renal anemia causes albumin to appear in the urine. Marshall and Crane report more detailed studies on the composition of the urine after temporary anemia, and conclude that water, chloride and bicarbonate excretion are unchanged or increased, while urea, phosphate, sulphate, creatinine and ammonia excretion are decreased. On the basis of these findings Marshall and

Crane advance a combination theory of renal mechanism: glomerular filtration plus tubular absorption plus tubular secretion. The absorption as well as the secretion work by the tubules might be more depressed by anemia than the glomerular filtration. On this theory, the urine first secreted after a temporary renal anemia should be more dilute than the control urine. It is also possible that in the anemic kidney the urine retained in the tubules may be diluted by diffusion with the blood and lymph.

TABLE 2

Dogs. Veronal anesthesia. Change in osmotic pressure of the urine induced by temporary ligation of the renal artery

NUMBER OF EXPERIMENT	Δ OF URINE				
	60 minutes anesthesia	120 minutes anesthesia	Renal anemia, minutes	First urine after anemia	90 minutes after anemia
1	1.22	1.32	20	0.82	1.89
2	2.87	3.10	20	1.70	2.51
3	2.63	2.45	5	1.42	2.22
4	1.53	2.43	5	0.75	1.35
5	2.23	3.34	10	1.83	3.08
6	2.95	2.23	10	1.13	2.82
7	2.28	3.28	15	0.61	2.94
8	2.14	1.99	15	1.29	1.87
9	1.54	1.36	20	1.13	2.80
10	1.28	1.62	20	2.21	1.55
11	2.22	2.71	20	2.93	2.56
12	1.22	1.62	20	0.82	2.12
13	2.56	1.81	15	1.11	2.34
14	0.31	0.61	15	0.51	0.76
15	1.77	1.82	10	1.52	1.43
16	1.45	2.90	5	0.63	2.00
17	1.23	1.23	5	2.56	1.74
18	2.93	2.61	5	3.10	0.60
19	2.63	2.14	5	1.15	1.33

To test this possibility we carried out a new series of renal occlusion experiments on dogs under veronal anesthesia, making freezing point determinations of the urine. It seemed important in this series to run controls on the anesthesia itself as a possible influence on the concentration of the urine in such prolonged experiments. Hence we usually made freezing point determinations on samples of urine after 1 and after 2 hours under veronal, on the first 2 to 5 cc. collected after the anemic anuria, and on the sample collected 1½ hours after the renal anemia.

The results are given in table 2. There are considerable variations in the urine samples in many of the individual experiments but the urine first secreted after a period of anemia and subsequent anuria seems clearly

more dilute in 13 out of the 19 experiments (nos. 1 to 9, 12 to 14, 16, 19). In experiments 17 and 18 the anemia seems to have increased temporarily the osmotic concentration of the urine, while no definite osmotic change can be made out in experiments 10, 11 and 14, in view of the fluctuations in the concentration in the other samples of urine.

We may therefore conclude that there is a definite tendency to produce a urine of lower than normal osmotic pressure for a short period after 5 to 20 minutes of anemia of the kidney, and the subsequent anuria. This seems in line with the report of Marshall and Crane that the elimination of urea, ammonia, phosphates, sulphates and creatinine is decreased by temporary renal anemia.

The interpretation of this urine change by anemia is not clear at present. There may be failure to secrete certain substances, as indicated in the work of Marshall and Crane. There may be decreased absorption of the glomerular filtrate water by the anemic tubules; or the anemia may so injure the vascular and the renal epithelium so that pathological diffusion relations come into play between the urine and the blood and lymph plasma.

3. The vascular spasm induced in the kidney by temporary anemia. It seems singular, in view of the important rôle that the anemic anuria has played in theories of renal mechanism, that practically no studies seem to have been made of the condition of the renal circulation following temporary renal anemia.

In our experiments we found the Roy kidney oncometer too cumbersome and difficult to use, if we wished to avoid tension on the renal pedicle and injury to the kidney. We therefore fixed a delicate rubber balloon in each half of the outer shell of the oncometer, the balloon being connected with a rubber tube through an opening in the end of the oncometer. The rubber balloons were connected with a water manometer of about 1 cm. diameter and the balloons were inflated with a pressure of about 5 cm. of water. This contrivance seems a very convenient kidney plethysmograph.

The kidney was isolated as much as possible from fat. All the small blood vessels to the capsule were ligated and sectioned so that the only vascular connections of the kidney remaining were the main renal artery and vein. The latter were isolated from each other and from the perivascular nerve plexus, so that the artery could be clamped without touching the renal nerves or pressing on the renal vein. In some experiments we sectioned the renal nerves before proceeding with the anemia experiments. The dogs were under veronal anesthesia, with a very slight amount of ether in a few of the experiments. The ureters were left intact. No attempt was made to record the urine flow. Records of the general arterial pressure were taken from the carotid artery.

In this way anemia of the kidney by clamping the renal artery for 5, 10 and 15 minutes was produced and the kidney volume studied on releasing the artery. Records of typical experiments are reproduced in figures 1, 2 and 3. The results may be summarized as follows:

1. The kidney shrinks at first rapidly, then very gradually on occlusion of the renal artery. The gradual shrinking may continue for the entire period of a 5- or 10-minute occlusion. This gradual reduction in kidney volume is probably caused by gradual vascular constriction in the kidney, thus forcing some of the retained blood into the vena cava.

2. When the clamp on the renal artery is released there is a sudden increase in the kidney volume. In the case of the 5-minute anemia experiments this sudden increase in renal volume may for a brief moment equal that of the control period, but usually it is less than the control volume. In the 10- and 15-minute anemia periods the initial and rapid kidney expansion never reached the control level. Then follows a period

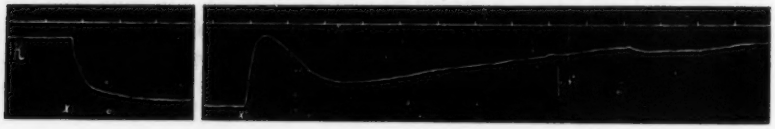

Fig. 1. *K*, plethysmograph record of the kidney; *X*, occlusion of renal artery (5 minutes); *X'*, release of renal artery. Time, minutes. Showing prolonged spasm of the kidney blood vessels following the anemia.

of gradual decrease of the kidney volume followed by a more gradual relaxation, this phase taking from 10 to 20 minutes, before the control level of kidney volume is reached. In general the longer the renal anemia, the longer the period of post-anemia vascular spasm of the kidney but there are many exceptions to this. In one experiment (20-minute renal anemia) the kidney showed marked but somewhat periodic spasm for over 1 hour on releasing the renal artery. These post-anemia changes in renal volume are not accounted for by changes in general blood pressure (figs. 2, 3). They must accordingly be due to local spasms in the renal vascular system.

3. In some of our experiments releasing the renal artery is followed by a slight and transitory (3 to 5 minutes) lowering of the general arterial blood pressure (fig. 2). This may be due to depressor substances developed in and washed out of the anemic organ.

4. We are unable, at present, to account for these post-anemic changes in renal volume on any other basis than vascular spasm. They are not due to long vasoconstrictor reflexes, as they are present in the denervated kidney. Hence we conclude that anemia of the kidney alters the local vascular mechanism in such ways that on reëstablishing the circu-

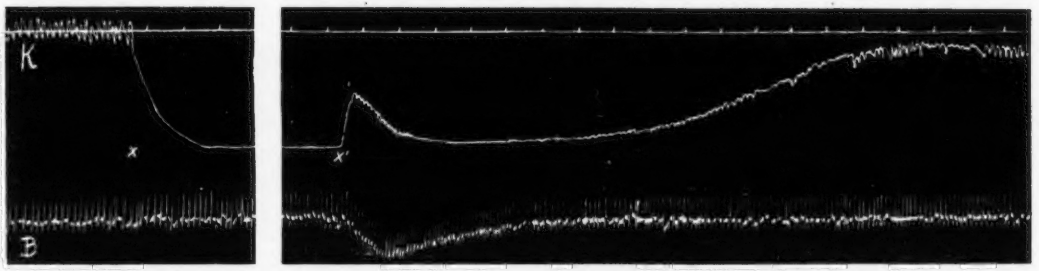

Fig. 2. *K*, plethysmograph record of the kidney; *B*, carotid blood pressure; *X*, occlusion of renal artery (10 minutes); *X'*, release of renal artery. Time, minutes. Showing post-anemic spasm of the kidney blood vessels.

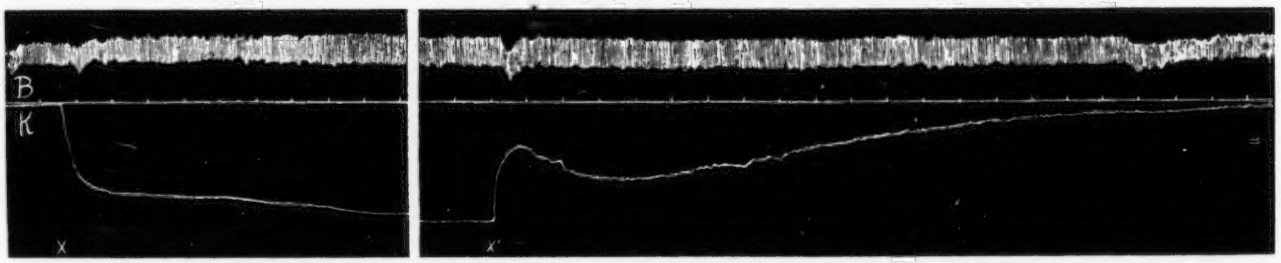

Fig. 3. *K*, plethysmograph record of the kidney; *B*, carotid blood pressure; *X*, clamping of renal artery (15 minutes); *X'*, release of renal artery. Time, minutes. Showing post-anemic spasm of the kidney blood vessels.

lation the blood vessels (probably both arteries, capillaries and veins) pass into prolonged spastic contractions, a degree of contraction greater than that induced by the anemia during the anemia period itself, as indicated by the initial increase in renal volume for a minute or two on releasing the occluded renal artery.

5. Can this post-anemic contracture of the renal blood vessels account for the usual post-anemic anuria? It seems well established that lowering of the general arterial pressure to about 60 mm. Hg induces anuria; and that decreasing the volume of blood going through the kidney decreases the output of urine. It is obvious that the marked renal spasm above described will decrease the volume of blood going through the kidney and if, as seems probable, the renal arterioles take part in the spasm, there will also be a decrease in the capillary pressure in the kidney. This would decrease filtration, if filtration is a factor in normal urine formation. It follows, therefore, that the post-anemic vascular spasm in the kidney by itself explains the post-anemic anuria. But it should be noted that in only one case have we observed a vascular spasm maintained for the length of time that one may produce anuria by a 20-minute clamping of the renal artery. The function of the renal epithelium may also be impaired by anemia, as indicated by the post-anemic qualitative changes in the urine. Either of these two factors may by itself cause temporary anuria, but working both in the same direction will evidently produce more marked effects.

6. In view of these new aspects of the prolonged effects of renal anemia on the renal vascular system, it would seem that the arguments anent the renal mechanism based on the prolonged effects of clamping the renal artery are not cogent, as they have been based on inadequately analyzed facts. The post-anemic anuria induced by clamping of the renal artery argues neither for nor against the glomerular filtration theory, until it has been shown that the anuria outlasts the spasm of the renal vessels. It is otherwise with the anuria caused during the clamping of the renal vein, or following release of the clamped vein. Clamping the renal vein for 10 to 15 minutes induces some spasm in the kidney blood vessels when the clamp is removed, but it is not yet clear that this spasm is either marked enough or prolonged enough to completely account for the anuria. The anemia of the kidney on clamping the renal vein for 5 to 15 minutes is obviously less complete than that induced by corresponding occlusion of the renal artery. The physical state of the renal vessels during the anemia is also very different in the two conditions.

CONCLUSIONS

1. Under conditions of as nearly normal blood composition, circulatory conditions and renal activity as the experimental necessities permit, we find that occlusion of the renal artery or the renal vein induces anuria not only during the period of anemia, but for varying periods following the release of the clamped vessels. This is in agreement with the original observations of Hermann, but contrary to the results of Marshall and Crane, who found no post-anemic anuria when diuresis is induced by establishing hydremia (10 per cent NaCl).

2. There is a distinct tendency to production of urine with lower than normal osmotic pressure for a period following the complete anuria. It is pointed out that this fact argues neither for nor against the glomerular filtration hypothesis.

3. Temporary anemia of the kidney induced by clamping the renal artery induces a prolonged spasm or contracture in the renal vessels on releasing the artery. This vascular spasm is of a degree and duration to account for some if not all of the anemic anuria. But parallel anemic injury to the renal and the vascular epithelium is not excluded as a factor both in the post-anemic anuria and in the qualitative changes in the urine produced by the kidney during recovery from complete anemia.

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STUDIES ON THE VISCERAL SENSORY NERVOUS SYSTEM

XV. The Motor Rhythm in the Isolated Lung of the Turtle (Chrysemus cinereus and Clemmys gutta)

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The observations of Carlson and Luckhardt (2) on the motor mechanism of the lungs in amphibia showed that in this group the motor tissues in the lung have a persistent peripheral automatism, like the heart, an automatism normally repressed or controlled by *inhibitory* nervous action from the central nervous system, while in turtles and snakes the lung contractions associated with the respiratory rhythm is primarily due to *motor* innervation via the vagi nerves. In this respect the reptilian lung appears to have differentiated in the same line as the upper end of the vertebrate esophagus, although the musculature of the reptilian lung is still of the smooth type, and a ganglionic nerve plexus similar to that in the gut is present. Carlson and Luckhardt noted a feeble tonus rhythm in occasional preparations after the turtle lungs had been completely severed from the central nervous system. Fano and Fassola (1) had previously reported similar findings on the isolated lung of the European turtle (Emys europaea).

The present work was undertaken with the view of definitely establishing the presence or absence of a peripheral motor automatism as a physiologic mechanism or factor in the lungs of the turtle. If such peripheral automatism exists, this probably would require the existence of inhibitory nervous connections with the central nervous system for its control or coördination with the respiratory rhythm. Carlson and Luckhardt were unable to secure definite evidence of extrinsic inhibitory nerves to the turtles lungs. If the lungs of the turtle are in an atonic condition for some time after section of the vagi, inhibitory nerve action could, of course, not be revealed, at least by the registration of the tonus state of the lungs.

Experimental methods. Turtles in the best physiological conditions were used, as Carlson and Luckhardt found that turtles in poor condition show feeble or no lung contractions even when the pulmonary vagi remain connected with the central nervous system. Some experiments were

run with lung left in situ, but isolated as much as possible from the adjoining tissues. In these experiments the brain and spinal cord were pithed so as to eliminate all central nervous factors. This preparation has the advantage of the maintenance of some circulation of blood through the lungs. But since in most cases the contractions of the isolated lung are both feeble and irregular, one must exclude all such factors as variations in heart rhythm, tension from gut contractions, etc., on the lung record. We accordingly made most of our observations on the lung removed from the body and kept in various types of moist chambers. In isolating the lung direct trauma must be avoided, and it is best not to touch the lung with the instruments, but to handle it by the bronchus and the connective tissue septa. A cannula was fixed in the bronchus and connected with a small bore water manometer for recording of the lung contractions. The isolated lung was placed on glass in a moist chamber and covered with a single layer of gauze moistened with turtle serum or Ringer solution. In one series of experiments the lungs were distended with a positive pressure of 3 to 4 cm. of water. In another series the lungs were distended by producing 3 to 4 cm. (water) negative pressure outside the lungs, to duplicate the physical state of the lungs in the intact animal.

It is well known that the turtle tissues, including the central nervous system, are very resistant to starvation, anemia and lack of oxygen. In all probability the lung tissues, in our method of experimentation, received sufficient respiratory exchange from the air inside and outside the lungs. Perfusion of the isolated turtle lung with blood or Ringer solution is feasible, but this was not attempted in the present series. The experiments were run at room temperatures.

Results. 1. Isolated lungs from turtles in good condition practically always show slow periodic contractions, at times quite regular, but usually irregular. These contractions continue for one to four days in carefully kept preparations. Occasionally marked tonus contractions each period covering ten minutes or more are seen (fig. 1, VIII). But the usual type of contractions is shown in figure 1, V to VII. It is therefore evident that the turtle lung has local automaticity, in addition to the rhythm induced from the central nervous system. The individual contractions of the local rhythm do not reach the maximum of the contractions induced via the vagi motor system. This may be due to lack of local coördination, that is, all the motor elements not being involved in each contraction, or we may even have some parts of the lung contracting while other parts are relaxing.

2. The degree of coördination of the various parts of the lung in this local automatism is difficult to determine from the character of the tracings. Some of the records are sufficiently regular to indicate local coördination of the entire lung. In other cases the types of irregularity

indicate different rates of contractions in different regions of the lungs. The lack of coördination may be due to the artificial state of the lung in these experiments, in the same way that fibrillation of the heart is caused

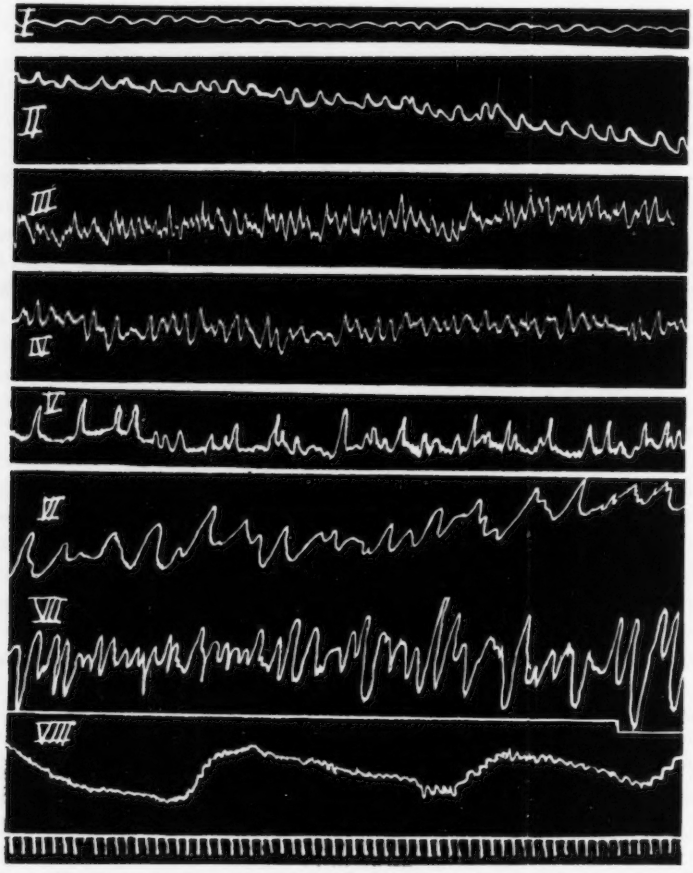

Fig. 1. Tracings showing types of rhythmic contractions of the isolated and excised lung of the turtle (crysemus cinereus and clemmys gutta). Water manometer, magnified by lever. Because of the delicate lines traced by the recording lever it was necessary to retrace lines on records I, II, V to VIII for reproduction. Time, minutes.

by depression of conduction in the heart tissues. In this connection it may be recalled that Carlson and Luckhardt reported complete coordination of the local automatism in the amphibian lung, the entire lung being in the state of contraction or in the state of relaxation at the same

time. But after local lung injury, or under conditions less physiological, even the amphibian lung may show lack of perfect coördination of the peripheral motor system. This recalls similar absence of coördination of the isolated stomach and intestines under bad experimental conditions.

Direct inspection of the isolated turtle lung does not help us decide the question of local coördination, for the contractions are too slow and feeble to be accurately followed by the eye.

The present experiments indicate, but do not demonstrate, the coördination of the local motor rhythm in the reptilian lung.

3. The rhythm of the isolated lung appears to be more continuous than that of the lung in situ and connected with the central nervous system, as shown by Carlson and Luckhardt. This indicates an inhibitory control of the local automatism in the intact animal. But the contraction force of the local lung automatism is very feeble in comparison with that of the isolated turtle esophagus. This difference does not seem to be one of quantity of musculature, for the turtle lung is capable of very powerful contractions on stimulation of the vagi or the medulla. The present observations thus seem to fall in line with the generalization of Carlson and Luckhardt (2) that in the reptilia the lung and the esophagus have differentiated very unequally from the assumed primitive local automatism and predominantly inhibitory control from the central nervous system, while the lung has predominantly, if not exclusively, motor control from the medulla, parallel with the character of the local automatism.

SUMMARY

1. The isolated lung of the turtle exhibits rhythmic contractions, continuing for days. This is true both when the lung is moderately distended by positive pressure, or distended within the physiological limits by reduced pressure outside the lungs.

2. In some cases the local rhythm is regular enough to indicate local coördination of the entire lung motor mechanism.

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THE FUNDAMENTAL RHYTHM OF THE HEIDENHAIN POUCH MOVEMENTS AND THEIR REFLEX MODIFICATIONS

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Practically all of the experimental work on separated stomach pouches has been done with reference to secretory function. The only study of motor activity in such a preparation, so far as we can find, was made by Carlson, Orr and McGrath (1). They used Pavlov pouch animals, making simultaneous records of the contractions of the pouch and of the main stomach. They found a certain degree of parallelism between the two, depending on the width of the connection left at the original operation. They state, however, "when the muscularis and myenteric isthmus joining the main and the accessory is relatively narrow, the two stomachs exhibit a complete independence of the hunger contractions, even to the point of vigorous activity of the one during quiescence of the other" (2). These statements referred to the hunger movements only. Their work was not extended to the Heidenhain pouch, which is completely cut off from the main stomach.

The present study was undertaken with the purpose of determining the type of movements in the Heidenhain pouch and their relation to those of the main stomach. In this preparation the myenteric nervous connections are all severed and the vagal fibers also, according to the common conception. Numerous small nerves, however, still reach the pouch through the attached mesentery and along the blood vessels. It seemed possible to us that these nerves, whatever their source, might furnish paths for reflexes from the stomach to the pouch or vice versa.

METHODS. The animals used were dogs. The pouch was cut off from the greater curvature of the stomach, the line of incision being essentially in the same position as in the Pavlov operation except that it was prolonged so as to sever the pouch completely from the stomach. The method of closing the incisions and of making the fistula differs in no essential respect from that used in the Pavlov operation. In a pouch so prepared the sole blood supply is by way of the left gastro-epiploic artery and vein.

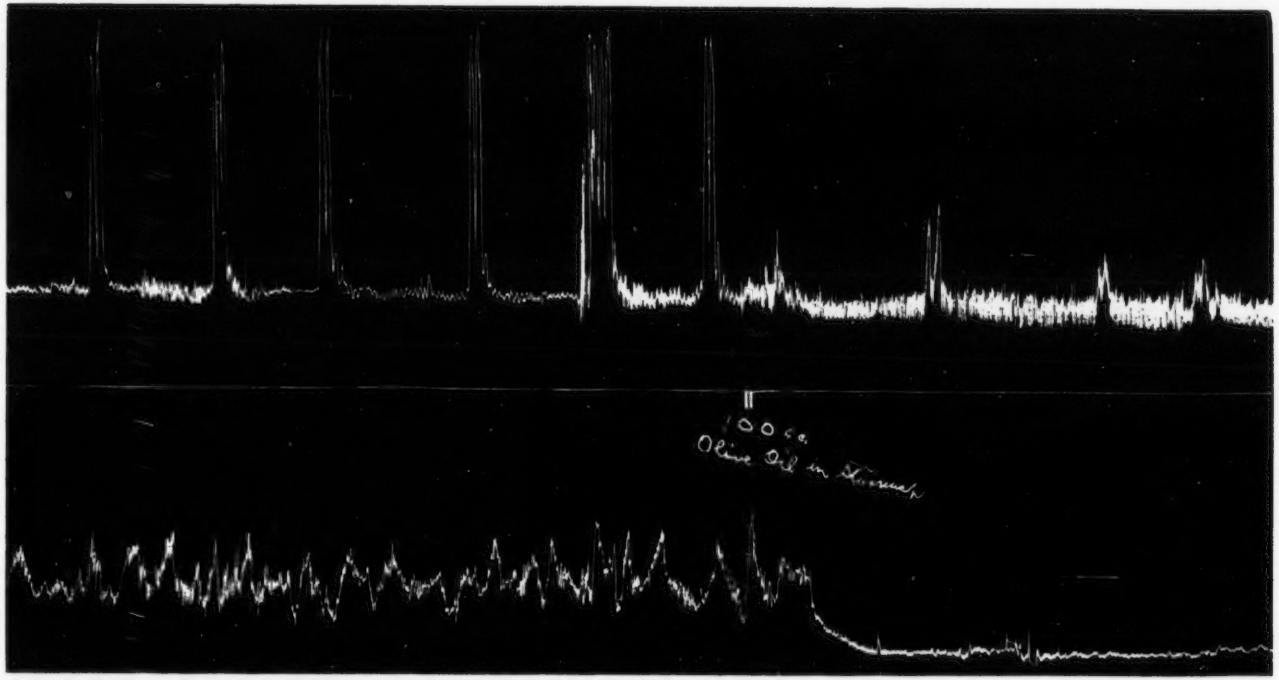

Fig. 1. Dog. Simultaneous water manometer records from the Heidenhain pouch (upper curve) and the stomach (lower curve) 24 hours after feeding. At the left of tracing are shown the typical periodic contractions in the pouch while the stomach is showing hunger contractions. The contractions in both pouch and stomach are inhibited by putting 100 cc. of olive oil into the main stomach through a stomach tube. Pressure in stomach balloon = 5 cm. water; pressure in pouch balloon = 3 cm. water. Total time of tracing, 60 minutes.

Our records were made by the balloon method. In some experiments simultaneous tracings were made from the stomach and the pouch; in others the tracing was made only from the pouch. The stomach balloon (condom) was introduced by way of the mouth and esophagus. At first it was necessary to push the balloon down by means of a stiff rubber tube, but after a few experiments most of the animals would swallow it voluntarily. The tube leading from the stomach balloon to the manometer was protected from the animal's teeth by means of a rubber gag. A second small tube was usually inserted with the balloon for the injection of substances into the stomach. The pouch balloon, which was smaller, was attached over the end of a hard rubber tube. The tube was freely perforated inside the balloon. Strings attached to the other end of the tube were passed around the animal's body and tied in order to hold the balloon in place. It was necessary to use a device like this otherwise the contractions of the pouch would force the balloon out. Water manometers were used with both balloons. A soft pad was provided for the dogs to lie on and after a few experiments they would lie quietly for two or three hours while a tracing was being made. Tracings were made on five different dogs with Heidenhain pouches.

RESULTS. *1. Types of movement in the pouch.* In general the movements in the pouch may be divide into three types:

a. The most constant and characteristic type of movement is a series of contractions recurring at strikingly regular intervals. Each period includes from two to five contractions which usually appear as separate peaks without complete relaxation between. The interval between the periods varies from three to ten minutes with an average of about six minutes. The length of the interval and the strength of the contractions vary with different animals. They also vary from time to time in the same animal. These rhythmical periods seem to be essentially hunger contractions, for they are usually associated with hunger contractions in the stomach and are usually absent following the ingestion of a mixed meal. Also the intervals between the periods are shorter and the contractions are stronger as fasting is prolonged. Exceptions to these statements are mentioned below.

b. The second type of movement is a single contraction, irregular in occurrence and strength. These appear in the intervals between the periods described above. In general they also tend to become more frequent as fasting is prolonged.

c. There are slight variations in tonus at all times. The respiratory rhythm usually appears on the tracing also.

2. Relation of the movements in the pouch to those in the stomach. The empty stomach shows characteristic types of hunger contractions as have been fully described by Carlson (2). Simultaneous records of stomach

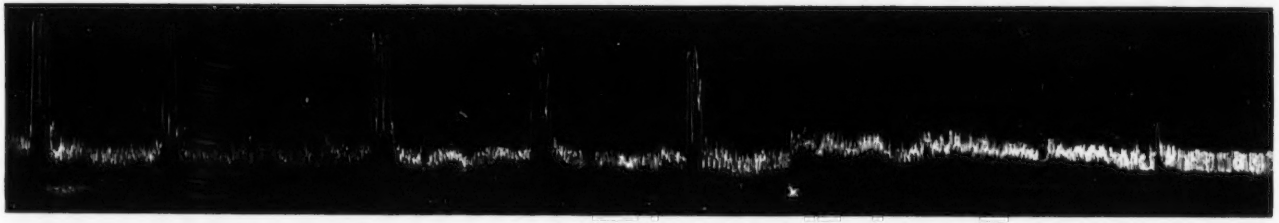

Fig. 2. Dog. Record of Heidenhain pouch movements 24 hours after feeding. At *X*, 150 cc. of milk were introduced into the main stomach through a stomach tube. The pouch contractions are inhibited. Pressure in pouch balloon = 3 cm.water. Total time of the tracing, 65 minutes.

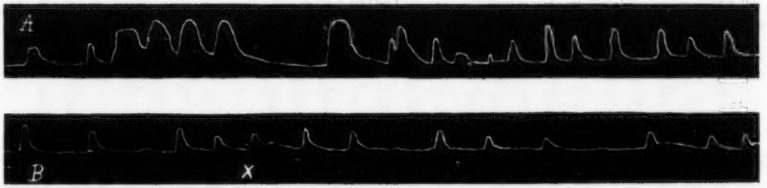

Fig. 3. Dog. The upper tracing, A, shows the irregular type of movement in the Heidenhain pouch, after stripping the mesentery to the pouch and cauterizing the blood vessels with $AgNO_3$. The lower tracing, B, is a part of the same tracing showing that 150 cc. of olive oil which were put into the main stomach at *X* did not affect the pouch movements. Pressure in the pouch balloon = 4 cm. water. Total time of each tracing, 16 minutes.

and pouch movements show that when hunger contractions are present in the stomach the rhythmical contractions described above are usually present in the pouch. There is no relation between individual contractions in the stomach and in the pouch. The pouch, however, may be quite active while the stomach is quiescent.

3. Experimental modifications of the pouch movements. When food is taken into the stomach which is showing hunger contractions the latter are inhibited, as shown by Carlson and others. We have not been able to inhibit the typical pouch contractions by putting substances into the pouch itself. The following liquids were put into the pouch through a small rubber tube which was inserted beside the balloon, milk, water, gastric juice, 0.3 per cent HCl, 10 per cent peptone solution, olive oil and 10 per cent dextrose solution.

Neither did any of these substances, put into the pouch, affect hunger contractions in the main stomach. Experiments similar to these were carried out on two Pavlov pouches with similar results. Therefore, no conclusion can be drawn as to a difference in nerve supply. The failure to obtain an inhibition may be due to the fact that there is only a small area of mucosa stimulated.

We have been able, however, to inhibit the typical pouch contractions by putting the following substances into the stomach: olive oil, milk, egg-yolk and meat scraps. The meat scraps were fed to the animal as he lay on the table, while a tracing was taken of the pouch motility only. The other substances were introduced by stomach tube in amounts of 100 cc. to 150 cc. None of the liquids gave uniform inhibition in amounts less than 100 cc.

The following substances introduced into the stomach had no effect whatever on the activity of the pouch: 10 per cent peptone solution, 10 per cent dextrose solution, water, agar solution, egg-white, gastric juice and 10 per cent alcohol. All of them produced at least a temporary inhibition in the stomach. The amounts used were 100 cc. to 150 cc.

4. Influence of nerves on the contractions of the pouch. We have not completed our study of reflexes to the pouch. But the following procedures were negative in effect: (*a*) Allowing the animal to gnaw bones; *b*, painful stimulation of the legs (pinching with forceps); (*c*) stimulation of the rectum by inserting a small test-tube brush; and (*d*) passage of a stomach tube. We did not try any more intense form of pain stimulus as the animals were never anesthetized. Some of the animals in the first experiments were excited and restless but this did not affect the rhythmical activity of the pouch.

In one animal the vagi nerves were sectioned above the diaphragm. This procedure did not affect in any way the rhythmical activity of the

pouch and inhibition from the stomach was as easily obtained after the operation as before. In another animal the greater and lesser splanchnics were severed on both sides, with equally negative results. These facts seem to indicate that the pathway involved in the inhibition is neither by way of the splanchnics nor the vagi. This point cannot be settled, however, until both sets of nerves are sectioned on the same animal. This we have not done so far.

In one animal we stripped away the mesentery entirely from the pouch leaving only the artery and vein previously mentioned. This procedure was without effect either on the movements or their reflex inhibition. Supposing the inhibition to be reflex, it thus became evident that at least some of the nerves concerned reached the pouch by way of the perivascular plexus of nerves.

In the attempt to settle this point two other animals were operated on as follows: In the first operation the entire mesentery was stripped away from the pouch leaving only the blood vessels. These pouches showed all the typical activity as previously described. In subsequent operations the blood vessels were carefully denuded and a ring cauterized around each by means of small crystals of silver nitrate. The pouch of one of these animals continued to show rhythmical contractions, but they were less constant and regular than before the operation. Olive oil put into the main stomach inhibited the contractions in three out of four experiments carried out after the second operation. In the fourth experiment it had no inhibitory effect. In the second pouch the rhythmical contractions were completely abolished after cauterizing the blood vessels. Weak irregular contractions, which were very similar to the second type previously described but which were more frequent, continued to appear. These were not affected by putting olive oil into the stomach. During a fasting period of eight days we carried out five experiments with this animal, the results in all being similar. We do not believe that the change could have been due to interference with the blood supply. The pouch continued to secrete and the color of the exposed mucosa was normal.

SUMMARY

1. The isolated stomach pouch (Heidenhain) shows a rhythmical type of movement. The periods of activity recur at intervals of from three to ten minutes. There is a certain degree of synchrony between the contraction periods in the empty stomach and in the isolated pouch.

2. We have not been able to modify the contractions by putting substances into the pouch.

3. We have not been able to modify the stomach hunger contractions by putting substances into the pouch.

4. The pouch contractions may be inhibited by putting certain substances, viz., olive oil, milk, egg-yolk and meat, into the main stomach. So far we have not produced the inhibition with any fat-free substance.

5. Our experiments indicate that this inhibition is probably a local reflex from the main stomach or the duodenum through the peripheral ganglia. This point cannot be settled until our experiments are extended and confirmed.

We wish to thank Dr. A. J. Carlson for valuable criticisms and advice in the course of this work and for assistance in the more difficult operations.

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BIOLOGICAL FOOD TESTS

V. The Biological Value of Almond Proteins and of Almond Oil

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The sweet almond, the fruit of *prunus communis*, is a nut that has been widely used in Asia and Europe for many centuries, but which has only recently been intensively cultivated in parts of the United States, particularly in California. About 98 per cent of the American almond crop is grown in California, where more than one hundred thousand acres of almond orchards have been set out. The number of bearing and non-bearing trees is estimated at seven millions, which should yield an annual crop of seventy million pounds by 1923.[1] In spite of this large domestic production, the United States imports large amounts of almonds. These imports for 1919–1920 reached a total of thirty-three million pounds, 80 per cent of which consisted of shelled nuts. The California crop has hitherto been marketed chiefly in the shell, about 25 per cent only as shelled nuts, but due to the recent increase in production, about tenfold since 1910, must now compete with the imported shelled nuts as well.

Until recent years the almond has been largely confined to luxury and holiday uses, but its increasing utilization by bakers and confectioners, in pastes and meals for diabetics, as salted nuts and nut butter, is significant of a wider future for this valuable food. It seems worth while therefore to review the evidence of its biological value and to determine if possible the nutritive quota which it may be expected to contribute to the American diet.

Digestibility. Several studies of the digestibility of nitrogen, fat and carbohydrate of almonds have been made. The first and most extensive is that of Jaffa (1) who found an average of 72 per cent utilization for protein, 85 per cent for fat, and 94 per cent for carbohydrate in six experiments using a mixed diet consisting of one or more fruits such as bananas, apples, dates, olives or oranges, and almonds. In these experiments the

[1] The figures concerning almond production and imports are quoted from statements made by officials of the California Almond Growers' Exchange and reported in the California Almond Growers' Exchange Bulletin 189, March 18, 1921.

almonds contributed 49 to 88 per cent of the protein fed, 5 to 25 per cent of the carbohydrate, and 89 to 95 per cent of the fat, except in the one experiment in which olives were used in the diet. These digestibility figures are therefore significant with reference to almonds only in regard to the fat. Jaffa nevertheless calls attention to the high digestibility of the almonds compared with walnuts, Brazil nuts and pecans used in parallel studies and reported in the same bulletin. Cajori (2) reported a coefficient of nitrogen utilization of 84 to 89 with three human subjects fed almonds and almond paste. He did not state however whether the almond was sole or chief source of nitrogen in the diet. Morgan and Heinz (3) found 75 and 87 per cent of the nitrogen absorbed by a human subject on a diet in which 60 per cent of the nitrogen was obtained from almond meal. Hoobler (4) found 90 per cent of the dietary nitrogen absorbed by two nursing mothers who were taking a mixed diet in which 60 per cent of the protein was from nuts.

Figures for the digestibility of the carbohydrate of almonds are not available, although Cajori (2) described a method for the determination of these data. Jaffa's figures for carbohydrate absorption apply to essentially a mixed diet of which the almond carbohydrate constituted only a small part. The nature and availability of almond carbohydrate, usually given as 17.3 per cent of the nut, are now under investigation in this laboratory.

Salt content of the almond. Ash analyses of the almond kernel are quoted by Atwater and Bryant (5).

The total amount of ash yielded by the nut is usually given as 2 to 2.5 per cent. Sherman (6) quotes the following as per cent of edible portion of almonds:

Ca	0.239	P	0.465
Mg	0.251	Cl	0.037
K	0.741	S	0.160
Na	0.019	Fe	0.0039

These figures are high for calcium, magnesium, phosphorus and iron when compared with those for other nuts and for most fruits and vegetables not excepting the dried legumes and cereals.

A recently published study by Rose and MacLeod (7) indicates almost as good utilization by human subjects of the calcium of almonds as of the calcium of milk.

Vitamin B in almonds. Only one study of the vitamin B content of almonds has been reported, that by Cajori (8), who found that four young rats grew normally when 2 grams of almonds were given daily in addition to a basal diet sufficient in all respects except as to vitamin B. When 1 gram only was given the recovery of the rats fed a vitamin-B-deficient

diet was not satisfactory although some improvement in growth was noted. In this respect the almond was found to be approximately one-half as potent as the pecan, English walnut or chestnut.

In view of data already available as noted here it was thought best to confine the study of the almond in this laboratory for the present to *a*, the extraction, identification and nutritive testing of the proteins of the almonds; and *b*, the study of the vitamin A value of almond oil.

a. The proteins of the almond. During recent years a considerable amount of attention has been directed toward the isolation, hydrolysis and biological testing of the proteins found in nuts. Perhaps the first of these investigations by modern methods was that of Osborne and Mendel (9) in 1915 on excelsin of the Brazil nut. Satisfactory growth was exhibited by young rats fed this substance as sole source of nitrogen in the diet. Hoobler (4), using the method of measurement of nitrogen balance in nursing women, found a diet of mixed nuts a surprisingly good source for the elaboration of milk proteins. Morgan and Heinz (3) who determined the biological value of almond meal by the usual minimum nitrogen feeding method calculated according to the formulas proposed by Thomas (10), placed the high value of 94 upon the united proteins of this nut.

Johns, Finks and Paul (11) isolated the globulin of the cocoanut and tested it and the protein of the cocoanut presscake by the usual rat-feeding technique. The presscake yielded excellent growth in the young animals at a protein level of 13.1 per cent, but the globulin had to be fed at the 18 per cent level. Cajori (8) showed by the same methods that the proteins of the English walnut, filbert, pine nut and almond all support satisfactory growth in young rats when they are the essential source of protein in the ration, and that they furnish suitable materials for the elaboration of milk in rats. Cajori (12) likewise fed the pecan nut successfully when the disturbing outer integument of this nut had been removed. Mignon (13) fed the globulin from English walnuts to mice with complete success at the 18 and 12 per cent levels, and with nearly normal results at the 9 per cent level as well.

With the exception of the work of Osborne and Mendel (9) with excelsin, of Johns, Finks and Paul (11) with cocoanut globulin, and of Mignon (13) with walnut globulin, no attempts at the feeding of isolated nut proteins have been recorded so far as is known to the writers.

The amino-acid content of nut proteins. A number of careful determinations of the nitrogen partition in isolated and purified nut proteins has been made. Osborne and Harris (14) worked out the distribution of basic amino-nitrogen in the globulins of almonds, Brazil nuts, filberts, black walnuts, English walnuts and butternuts, and concluded that the chief globulins of the three latter were similar if not identical. Osborne and Clapp (15) studied in more detail the products of hydrolysis of the chief

globulins of the almond and the Brazil nut, amandin and excelsin, and Nollau (16) using the Van Slyke method analyzed the nitrogen content of fat-free meal from black walnuts and pecans. Dowell and Menaul (17) made a similar study of the alkali-precipitated proteins of pecans and peanuts, Johns and others (18) of the isolated globulins of the peanut and cocoanut. Cajori (12) using the Van Slyke technique has carried out a similar study upon an isolated pecan globulin, and Mignon (13) upon the chief globulin of the English walnut. All of these investigations have shown that the nut globulins yield a relatively high proportion of basic amino-acids, although no uniformity in this respect is observed. The figures for lysine given by Osborne and Clapp, who used the method of Kossel and Kutscher, are notably lower than those obtained by later investigators who use the Van Slyke method. Thus amandin is reported by Osborne and Clapp to show only 0.72 per cent lysine, while Cajori using the Van Slyke method found 6.2 per cent of the same amino-acid in pecan globulin, and Mignon with the same method 10.61 per cent in walnut globulin. The amount of cystine recorded in these cases likewise varied considerably, a factor which must be carefully considered in any attempt at prediction of the nutritive value of proteins.

The investigation of almond proteins here reported was undertaken with the object of discovering the relative biological values of the chief globulin, probably the amandin of Osborne and Clapp, and of the remaining almond proteins.

A preliminary study showed that there probably exist two globulins and one albumin in the almond, and that 49 per cent of the total nitrogen of the fat-free almond meal is extractable by water, 27 per cent by 10 per cent sodium chloride solution and 27 per cent by 0.1 saturated ammonium sulfate solution. Careful determination of coagulation temperatures of these extracts was made in an attempt to establish the separate identities of the proteins present.

Coagulation temperatures of salt solution extracts of almond meal. Three extracts of the dried fat-free almond meal were made in addition to that with 10 per cent sodium chloride solution. These were with distilled water, followed by filtration and extraction of the residue with 0.01 saturated ammonium sulfate, a second filtration and extraction of the residue with 0.1 saturated ammonium sulfate. The water and ammonium sulfate solution extracts were heated in the usual manner for the determination of coagulation temperatures with results as shown in the second column of table 1. In order to eliminate the effect of varying concentrations of salts used in these extracts, the process was repeated after addition of enough solid ammonium sulfate to the 0.01 saturated extract to make it 0.1 saturated, and the dilution of the 0.1 saturated extract to make it 0.01 saturated ammonium sulfate solution. The results are recorded in col-

umns 3 and 4 of table 1. The dilution to 1 per cent in the case of the 0.1 saturated ammonium sulfate extract produced so weak a solution as to render unreliable the coagulation results, and these figures are therefore not included in table 1.

Similar coagulation temperatures were determined for the 10 per cent sodium chloride extract and for solutions of the chief globulin precipitated from it, but with filtration after each precipitation obtained during the

TABLE 1

Coagulation temperatures and precipitation of almond proteins extracted by ammonium sulfate solutions

1	2	3	4	5
PROTEIN EXTRACT	COAGULATION TEMPERATURE	COAGULATION TEMPERATURE IN 0.1 SATURATED SOLUTION AMMONIUM SULFATE	COAGULATION TEMPERATURE IN 0.01 SATURATED SOLUTION AMMONIUM SULFATE	PRECIPITATION
Water (extract A)	65–76	82–92	80–97	Saturated solution ammonium sulfate produced slight precipitation
0.01 saturated solution ammonium sulfate (extract B)	74–84	67–76		0.12 saturated solution ammonium sulfate produced precipitate
0.1 saturated solution ammonium sulfate (extract C)	84–90			0.4 saturated solution ammonium sulfate produced precipitate

heating. These results as shown in table 2 indicate the presence of two and possibly three proteins in the salt solution extract of almond meal. The conclusions which may be drawn from the results given in tables 1 and 2 are as follows:

1. The water soluble proteins, extract A, differ from those extracted with 0.1 saturated ammonium sulfate solution, extract C.

2. Extract B, that obtained with 0.01 saturated ammonium sulfate solution may differ from extract A and extract C, but its protein may be made up of a mixture of the two.

3. Extract D, that obtained with 10 per cent sodium chloride solution, contains proteins exhibiting two decidedly different coagulating points.

4. Of the proteins precipitated from extract D by a 0.33 saturated solution of ammonium sulfate, that coagulating at the higher temperature is considerably less soluble in either water or 10 per cent sodium chloride solution than the other.

5. There are present in the almond, *a*, an albumin soluble in water, precipitated by complete saturation with ammonium sulfate and coagulable at 55 to 65°; *b*, a globulin, soluble in 10 per cent sodium chloride solution and in 0.1 saturated ammonium sulfate solution, precipitated by 0.4 saturation with ammonium sulfate, and coagulable at 84 to 90°. This is the chief globulin of the almond and is probably identical with amandin. *c*, Possibly a second globulin, soluble in 0.01 saturated solution ammonium

TABLE 2

Coagulation temperatures of almond proteins extracted by 10 per cent sodium chloride solution

PROTEIN PRECIPITATION	FIRST CLOUDINESS	DECIDED TURBIDITY	PRECIPITATE SETTLING
10 per cent sodium chloride solution extract of fat-free almond meal (extract D)	45	50	55–60
	After first filtration:		
	60	75	84
	After second filtration:		
			90
Solution in water of purified almond protein extracted by 10 per cent sodium chloride solution (solution D)	55	65	70
	After filtration:		
	80		90
Almond protein precipitated from 10 per cent sodium chloride solution extract by 0.33 saturated ammonium sulfate dissolved in 10 per cent salt solution (solution D_1)	60	70	80
	After filtration:		
	90		
Filtrate from precipitate D_1	80		

sulfate, precipititated by 0.12 saturation with ammonium sulfate, and coagulable at 74 to 84°. This may be a mixture of the albumin and amandin. Samples of these three protein preparations purified by reprecipitation with ammonium sulfate are now being analyzed for elementary composition, and the partition of basic N as determined by the Van Slyke method. The results will be reported in a later paper.

The isolated protein used in the feeding experiments hereafter described is probably therefore a mixture of two globulins, although possibly identical with the substance called amandin by earlier investigators.

Preparation of the globulin. The globulin mixture used was prepared by the general method described by Johns, Finks and Gersdorff (18) for the isolation of cocoanut globulin. Five pound lots of shelled and blanched California almonds were ground to a buttery consistency in a food chop-

per, dried in the air, and part of the oil squeezed out of the pulp by means of a hand press. The residue was then thoroughly extracted with ether in a large Soxhlet extractor leaving a practically fat-free meal. This meal was soaked in 10 per cent sodium chloride solution for three to seven days, the liquor strained through muslin bags, and the residue again extracted with sodium chloride solution in the same way. After the combined liquor had been filtered through asbestos the opalescent filtrate was dialyzed for three days. The precipitated protein was then removed and the liquor dialyzed again for two days. This double period of dialysis was found to yield much larger quantities of protein than the seven-day period used by Johns. The precipitated globulin was washed with distilled water and with 50 per cent alcohol, suspended in absolute alcohol overnight, and then in absolute ether. It was then removed from the ether, dried in a current of air and ground to a fine grayish powder. The highest yield obtained was 117 grams of protein from five pounds of the shelled nuts. That this may be the maximum yield possible by this method of extraction seems probable in view of the results of the preliminary study already quoted.

The almond globulin obtained as described gave a positive test for tryptophane by the Hopkins-Cole method, and responded positively to all other ordinary protein color reactions. It showed 15.8 per cent nitrogen as determined by the Kjeldahl method. Osborne and Clapp (15) reported 19 per cent nitrogen for their amandin. If the latter figure is accepted as representing the purest form of amandin hitherto prepared, our globulin would assay 83.7 per cent amandin.

Preparation of residue. A few animals were fed the residue from the salt solution extraction of the almond meal. This residue was so strongly impregnated with salt that it had to be washed thoroughly with several portions of distilled water in order to make it acceptable to the animals. The washed residue was fat-free, contained 1.2 per cent nitrogen, corresponding to 7.5 per cent protein and 6 per cent of sodium chloride. Although tests for reducing sugar and for starch were negative there remained in the residue considerable amounts of indigestible carbohydrate which caused some diarrhea in the experimental animals. Further study of the nature of these carbohydrates of the almond is in progress in this laboratory.

Feeding experiments. Both mice and rats were used as experimental animals, the former because of their smaller food intake in the tests upon the globulin. The mice used were of the same strain, the growth rate of which had been intensively studied by Robertson and Ray (19), so that normal curves for comparison with our experimental results were easily accessible. The mice were caged and cared for in practically the same manner as that previously described from this laboratory for rats (20).

Diets used. In all cases a basal diet similar to that previously described (20) was used, with the protein to be tested substituted for the casein. Special care was exercised in the measurement of food intake. Nine per cent and 14 per cent almond globulin levels were tried but were speedily found to be inadequate. The protein test diets used were made up as follows:

Almond globulin diets, 9, 14 and 18 per cent protein

Almond globulin (15.8 per cent N)	18, 14, 9
Salt mixture (Osborne and Mendel, 1917)	4, 4, 4
Agar	1, 1, 1
Dextrin	57, 61, 66
Crisco	2, 2, 2
Butterfat	18, 18, 18

Dried brewery yeast, 100 mgm. daily per mouse

Almond meal diet, 17.2 per cent protein

Almond meal (6.9 per cent N)	40
Salt mixture	4
Dextrin	36
Agar	0
Crisco	2
Butterfat	18

Dried brewery yeast, 100 mgm. daily per mouse

Almond residue diet, 4.5 per cent protein

Washed almond residue (1.2 per cent N)	60
Salt mixture	4
Crisco	18
Butterfat	18

Dried brewery yeast, 100 mgm. daily per mouse, 300 mgm. daily per rat

The proportion of fat in the last diet was increased beyond that in the other two because it was recognized that the bulky almond residue provided little that was digestible beyond its protein content, and sufficient caloric intake could be assured only by addition of fat.

A number of litter mate controls in each group were fed the usual casein diet, with careful measurement of food intake.

Results of feeding experiments. As may be seen from the growth chart 1, the mice fed the isolated almond globulin at the 18 per cent level were distinctly subnormal in size at the end of four weeks' feeding. The mice fed the almond meal diet, representing the whole almond protein at practically the same level (17.2 per cent) achieved normal growth, as did also both the mice and the rats fed the residue diet, representing the nonglobulin protein of the almond, even at the low level of 4.5 per cent protein.

Discussion and critique of numerical protein efficiency measurement. Further comparison of the efficiency of these protein mixtures is offered in table 3 in which food intake and increase in body weight per gram of

protein eaten are shown. The latter figures may be compared with those of Osborne, Mendel and Ferry (21), who made similar studies on casein, lactalbumin and edestin, using various protein levels, but rats in all cases as the experimental animals. It is interesting to note that the highest protein efficiency recorded by them, 3.0 grams gain in the body weight on

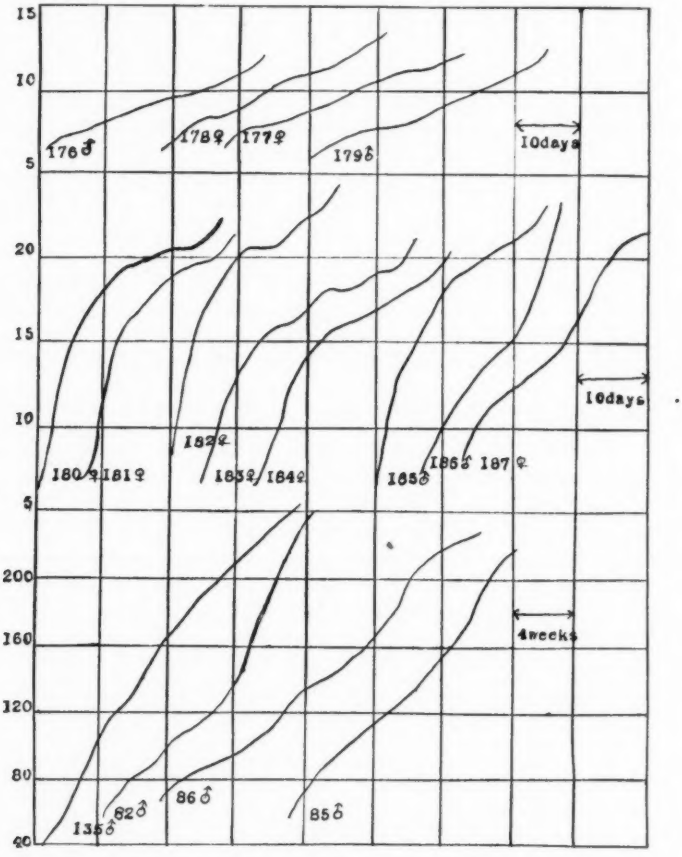

Chart 1. Illustrative growth curves of mice and rats fed almond proteins

Mice 176, 177, 178 and 179 were fed almond globulin at an 18 per cent level. Mice 180, 181, 182, 183, 184 and 185 were fed a diet containing 40 per cent fat-free almond meal providing 17.2 per cent almond proteins. Mice 186 and 187 were fed a diet containing 60 per cent washed salt extracted fat-free almond meal providing 4.5 per cent non-globulin almond proteins.

Rat 135 was a casein-fed control animal. Rats 82, 85 and 86 were fed the non-globulin almond proteins at a 4.5 per cent level similarly to mice 186 and 187.

TABLE 3

Growth of mice and rats on various almond protein fractions

MOUSE	DIET	DAYS OF FEEDING	INITIAL WEIGHT	FINAL WEIGHT	GAIN	WEIGHT ROBERTSON'S MICE SAME AGE	TOTAL FOOD INTAKE	PROTEIN INTAKE	GAIN IN WEIGHT PER GRAM PROTEIN	COMMENT
			grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	
9 ♀	9 per cent almond globulin	26	6.8	10.6	3.8	14.9	57.9	5.2	0.73	Growth about 25 per cent less than normal
10 ♀		26	7.0	11.6	4.6	14.9	56.7	5.1	0.90	
	Average			11.1	4.2	14.9	57.3	5.1	0.81	
3 ♂	14 per cent almond globulin	25	7.2	11.0	3.8	16.8	58.6	8.2	0.46	Growth about 35 per cent less than normal. (Average variability at this age 13.9 per cent)
4 ♂		25	7.3	10.7	3.4	16.8	60.7	8.5	0.40	
5 ♀		25	7.1	10.3	3.2	14.9	60.2	8.4	0.38	
7 ♀		25	6.6	10.0	3.4	14.9	61.9	8.6	0.39	
8 ♂		25	7.2	11.1	3.9	16.8	59.5	8.3	0.47	
	Average			10.6	3.5	16.0	60.1	8.4	0.42	
176 ♂	18 per cent almond globulin	28	7.0	12.5	5.5	16.8	89.6	15.9	0.34	Growth about 18 per cent less than normal
177 ♀		28	7.3	12.5	5.2	14.9	89.6	15.9	0.34	
178 ♀		28	8.0	14.4	6.4	14.9	90.0	16.0	0.40	
179 ♂		28	7.2	12.6	5.4	16.8	89.3	15.9	0.33	
	Average			13.0	5.6	15.8	89.6	15.9	0.35	
180 ♀	40 per cent almond meal (17.2 per cent mixed almond proteins)	30	7.1	16.8	9.7	14.9	84.1	14.4	0.67	Normal growth attained, 7 per cent above average
181 ♀		27	8.3	16.7	8.4	14.9	83.9	14.4	0.58	
182 ♀		27	8.3	19.0	10.7	14.9	84.5	14.5	0.73	
183 ♀		27	6.6	14.6	8.0	14.9	83.2	14.3	0.55	
184 ♀		28	5.1	13.5	8.4	14.9	83.2	14.3	0.58	
185 ♂		28	7.6	17.5	9.9	16.8	84.1	14.4	0.68	
	Average			16.3	9.2	15.2	83.8	14.4	0.63	

186♂ 187♀	60 per cent almond residue (4.5 per cent protein)	27 27	9.6 9.3	14.0 13.0	4.4 3.7	16.8 14.9	92.5 86.1	4.1 3.8	1.07 1.02	Growth 14 per cent below normal, just at lower limit of variability
	Average.....			13.5	4.0	15.8	89.3		1.04	
11 male mice	18 per cent casein (controls)	28	9.2	19.7	10.5	16.8	78	14.0	0.75	Growth excellent, 17 per cent above average
14 female mice	18 per cent casein (controls)	28	9.3	16.7	7.4	14.9	75	13.5	0.55	Growth excellent, 12 per cent above average
Average all control mice.....		28	9.2	18.0	8.8	15.7	76	13.6	0.64	
RAT										
82♂ 85♂ 86♂	60 per cent almond residue (4.5 per cent protein)	28 28 28	67 59 70	109 103 116	42 44 46		235 268 258	10.5 12.0 11.6	4.00 3.66 3.96	Growth practically normal
	Average.....		65	109	44		253	11.4	3.87	
9 male rats	17.4 per cent casein (Osborne, Mendel and Ferry (21))	28	64	119.5	50.5		187	32.5	1.74	

7.9 per cent lactalbumin over a period of twenty-eight days, is surpassed by our 3.87 grams increase in body weight per rat on 4.5 per cent non-globulin almond proteins.

It must be recognized in weighing the value of this method of comparing the efficiencies of proteins that, other things being equal, the advantage rests with low level protein diets and short experimental periods covering the earlier growth cycle of the animals, just following weaning time.

No earlier studies of gain in body weight by mice per gram of protein eaten are known to the writers except that made by Mignon (13) with English walnut proteins, so that only the almond proteins here used and the English walnut proteins can be compared in this manner. The striking nutritive advantage of the whole almond proteins and of the non-globulin proteins of the residue over the extracted globulins is evident, even though the period of feeding is short and the number of animals used small. The longer experimental periods used by Mignon, one hundred and twenty-five and one hundred and five days, account at least in part for the lower figures for increase of body weight per gram of protein eaten. The comparison given in table 4, however, of the growth of some of the almond-fed mice over a period of one hundred and ten and one hundred and seven days with Mignon's results for one hundred and twenty-five and one hundred and five days on walnut proteins indicates remarkable similarity in the effectiveness of these nut proteins when fed at the same level. Similar comparison of the growth of rats on these proteins cannot be made since the time intervals were not alike, one hundred and five and one hundred and twenty-five days on the walnut proteins, and thirty-seven and forty-four days on the almond proteins. Such a discrepancy invalidates all comparisons of the biological efficiency of proteins by this method, and makes obvious the necessity of a standard procedure as to kind of animal, nature of basal diet, length of experiment, normal growth attained, and level of protein feeding in all tests of this kind.

In the early work of Osborne and Mendel (22) various periods were used, fifty-six days, seventy-seven days, and an indefinite period, "till the animals failed to eat satisfactorily." The amount of food offered the animals was restricted, however, in the attempt to obtain equal growth increments in the same periods and upon like food intake but varying levels of different proteins. This method of comparison of protein efficiencies was cumbersome and artificial, and was discarded by its proponents in favor of the feeding of unrestricted amounts of food containing different percentages of the same protein for four-week periods, with observation of gains in body weight per gram of protein fed. Thus the concentration of protein providing for most rapid increments of body weight could be detected. For example, lactalbumin at a 16.2 per cent level yielded a

smaller gain in weight per gram, 1.97 grams, than at a 7.9 per cent level, 3.01 grams, probably because of the excess protein provided above the minimum needed. On the other hand, rations containing less than 7.9 per cent lactalbumin produced smaller gains per gram of protein; for example, 1.43 grams at a 3.3 per cent level, because of actual inadequacy of protein supply. As was indicated by Mignon, however, other factors than gross average gain of the animals must be considered in evaluating such results. The variability of gains made at the lower levels, the general

TABLE 4

Comparison of growth of mice per gram of almond and walnut proteins

MOUSE	DIET	LENGTH OF EXPERIMENT	TOTAL FOOD INTAKE	PROTEIN INTAKE	GAIN IN BODY WEIGHT	GAIN IN BODY WEIGHT PER GRAM OF PROTEIN
		days	*grams*	*per cent*	*grams*	*grams*
80	Mixed almond proteins, (40 per cent almond meal)	110	365	17.2	14.3	0.22
81		110	378	17.2	14.9	0.23
82		110	405	17.2	14.3	0.20
83		110	375	17.2	12.7	0.19
84		107	381	17.2	13.0	0.20
85		107	396	17.2	15.6	0.22
Average			383	17.2	14.3	0.21
	(Mignon)					
3 mice	Mixed walnut proteins	125	356	18.0	14.0	0.22
2 mice	Mixed walnut proteins	125	363	12.0	16.3	0.44
2 mice	Mixed walnut proteins	125	345	9.0	14.4	0.46
3 mice	Walnut globulin	125	368	18.0	18.8	0.28
3 mice	Walnut globulin	125	328	12.0	16.5	0.42
3 mice	Walnut globulin	125	332	9.0	14.9	0.50
3 mice	Non-globulin walnut residue proteins	105	374	18.0	16.4	0.22

condition of the animals, sleekness of fur, activity, bone, tooth and organ tissue conditions as shown on autopsy, should also be taken into account. Another factor of importance in affecting the numerical results obtained in these comparisons is the adequacy of vitamin content, and proportion and availability of carbohydrate of the basal ration used.

Unless normal growth is attained during the period of observation a numerical expression of protein potency cannot be found by the process of dividing the total protein intake by the gain in body weight. For example, two mice fed almond globulin at a 9 per cent level, as shown in table 3, made an average gain in body weight of 4.2 grams in twenty-eight days following weaning, on a protein intake of 5.1 grams. This might

be expressed as a gain of 0.81 gram per gram of protein, a figure which compares well with that similarly calculated for casein at an 18 per cent level. In the latter case however full growth was attained, a gain in body weight of 8.8 grams on protein intake of 13.6 grams, or 0.64 gram increase per gram of protein eaten. The casein-fed mice come well within the zone of variability for normal mice of their age on mixed diet as recorded by Robertson and Ray (19), but the almond globulin-fed animals fall considerably below. Both the almond meal-fed and almond residue-fed mice show weights at the end of the experimental period which compare favorably with those quoted by Robertson for this same strain of animals. Inbreeding the stock given this laboratory by Robertson, with discarding of imperfect or stunted animals, has produced an excellently consistent type of mouse, which shows as little variation in growth, fertility and other characteristics as it is reasonable to expect in so highly organized an animal. All properly nourished specimens of this strain should therefore fall well within the limits for normal mice found by Robertson and Ray. Under this proviso then numerical figures for the biological value of the almond globulin calculated by the method proposed by Osborne, Mendel and Ferry are not significant, but those for the proteins of almond residue and almond meal are significant. Similar reasoning should apply to the work with rats. The three rats fed the almond residue proteins at the 4.5 per cent level attained practically normal growth in the twenty-eight-day period at the rate of 3.87 grams increase of body weight for each gram of protein eaten. With the final weight of these rats but little below that shown by the normal albumin and casein-fed rats of Osborne, Mendel and Ferry it is evident that the utilization of almond residue proteins when expressed by this method is considerably better than that indicated by any figures quoted by the above-mentioned authors for either casein or lactalbumin.

SUMMARY OF EXPERIMENTS UPON ALMOND PROTEINS: 1. Differences shown by water and salt extracts in coagulation temperatures, solubility, and precipitation by ammonium sulfate indicate the probable presence of an albumin and one or two globulins in the almond. Forty-nine per cent of the nitrogen of almonds is extractable by distilled water, 27 per cent by 0.1 saturated ammonium sulfate solution, and 27 per cent by 10 per cent sodium chloride solution.

2. The isolated globulin of the almond prepared by dialysis of the 10 per cent sodium chloride solution extract, does not support normal growth of mice when fed as sole dietary protein at 9, 14 and 18 per cent levels of intake.

3. The mixed almond proteins obtained by removing the oil from ground blanched almonds with U. S. P. ether, when fed at a 17.2 per cent level sufficed for normal growth of mice.

4. The washed residue left after the fat-free almond meal had been extracted with 10 per cent salt solution to remove the globulin, when fed to rats and mice as the sole source of dietary protein at a 4.5 per cent level permitted almost normal growth.

5. A criticism is offered of the method of comparison of the biological value of proteins by expressing the increase in body weight of animals per gram of protein eaten as measured for short intervals. It is suggested that several factors not always controlled may affect these figures, and that the degree of normally expected growth attained during the experimental period is particularly important in this respect.

b. Vitamin A in almond oil. A considerable amount of interest is attached to the determination of the vitamin A content of nut oils because of their increasing use in the manufacture of margarins. Halliburton and Drummond (23) tested cocoanut oil and nut butters for this property and concluded that these substances were practically devoid of value as sources of this vitamin. Coward and Drummond (24) examined Brazil nuts, Barcelona nuts, peanuts, walnuts, almonds and butternuts as to their vitamin A value, and found that approximately 1 gram of nut per day in no case furnished sufficient vitamin to restore to normal condition young rats which had ceased to grow on a vitamin-A-deficient basal diet. Only three animals were used for each nut tested during the fourteen-day trial, and among these considerable variation in initial weight may be observed. In one of the earlier studies which led to the discovery of the existence of vitamin A, Osborne and Mendel (25) used a diet containing 18 per cent cold-pressed almond oil in an attempt to restore growth in failing rats. No recovery was obtained with this diet, although under the same circumstances butter and cod liver oil were found to bring about rapid resumption of normal growth. The basal diet used in these experiments was not, however, vitamin-free in the sense understood at present. In a recent preliminary communication Rose and MacLeod (30) report experiments with the whole almond used as source of vitamin A. They use diets containing 81 per cent of whole almond and of almond press-cake from which part of the oil had been removed. On both these diets the animals failed to grow. A purified basal diet was then used, containing meat residue, starch, lard, salts and yeast, with 3, 5, 10 and 15 per cent additions of the blanched almond diet made when growth of the animals had ceased due to the vitamin A deficiency of the basal diet. In all cases brief intervals of resumption of growth were followed by decline. They conclude that there must be some interfering substance in the kernel of the almond, and that this nut is at the same time a fairly rich source of vitamin A. No protocols have as yet been published. Our experience with diets containing 40 and 60 per cent of almond meal and almond residue does not bear out the theory that the almond contains any substance which

inhibits growth of either rats or mice when the diet is complete with reference to both vitamins A and B. We were particularly interested in the vitamin A content of the separated almond oil, a substance which had not been studied except in the early work of Osborne and Mendel mentioned above.

Feeding experiments with almond oil. Both mice and rats were used to test the almond oil for its effect upon inhibited growth and other symptoms resulting from the use of vitamin A free diet, and mice were used in addition to observe the effect of such supplemented diets upon fertility. The oil was given as an integral part of the ration in one set of experiments, and administered separately from the basal diet in another. Both curative

TABLE 5

Growth of mice on almond oil as source of vitamin A

GROUP	NUMBER OF MICE	INITIAL AGE	PERIOD OF OBSERVATION	INITIAL WEIGHT	FINAL WEIGHT	GAIN	AVERAGE DAILY INTAKE		METHOD
							Basal diet	Almond oil	
		days	*days*	*grams*	*grams*	*grams*	*grams*	*grams*	
1	2	29	74	7.8	19.9	12.1	2.5	0.25	Preventive
2	6	26	76	6.6	19.8	13.2	2.8	0.42	Preventive
3	4	48	63	14.1	20.0	5.9		0.2	Curative
4	3	50	63	14.7	20.2	5.5		0.2	Curative
	Controls							Butter fat	
5	9	48	63	15.6	22.0	6.4		0.30	Preventive
6	9	28	76	9.1	21.4	12.3	2.9	0.14	Preventive

and prophylactic types of experiments were carried out with the oil. In table 5 the results obtained with mice are summarized.

It is evident to us from these and other studies that the mouse is not well adapted for use in experiments dealing with vitamin A deficiency. In no case have we observed eye inflammation in mice kept on vitamin-A-free diet although these animals after relatively long intervals, ten to twelve weeks in most cases, became progressively weaker and died if the missing vitamin were not added to their food. Upon precisely the same basal diet all our rats when taken at weaning show the well-known xerophthalmia in twenty-five to thirty days. The results shown in table 5 would appear to indicate that almond oil administered to young mice in quantities as small as 0.2 gram per day in addition to a basal diet sufficient in all respects except as to vitamin A provides for normal growth quite as well as do similar quantities of butterfat. It is probable, however, that a favorable conclusion as to the value of almond oil is not justified in view of the opposite results obtained with rats under similar conditions, as shown in

table 6. The remarkable growth of mice upon diets undoubtedly deficient in vitamin A noted in this and other studies in this laboratory, may be due to a low vitamin A need in these animals or to an unusual capacity

TABLE 6

Growth of rats on almond oil as source of vitamin A

NUMBER OF RAT	PERIOD OF OBSERVATION	INITIAL WEIGHT	FINAL WEIGHT	GAIN	AVERAGE WEEKLY FOOD INTAKE	AVERAGE WEEKLY OIL INTAKE	METHOD	COMMENT
	weeks	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*		
38	15	81	285	206	92	9.2	Preventive (10 per cent oil)	Decline postponed probably because of size of animal at the beginning
39	15	88	284	196	80	12.0	Preventive (10 and 20 per cent oil)	Decline arrested by administration of butter fat in the 13th week
45	13	105	236	131	98	12.2†	Curative	
40	9*	47	146	99	67	6.7	Preventive (10 per cent oil)	Decline and death
48	11*	54	132	78	63	6.3	Preventive (10 per cent oil)	Decline and death
41	12*	61	148	87	71	12.1†	Curative	Decline and death
49	10*	54	144	90	73	12.7†	Curative	Decline and death
						Butter fat		
50 (control on 5 per cent butter fat)	15	70	347	277	95	4.7	Preventive	Normal growth

* Dead.

† Last 4 weeks.

for storing excess supplies of this vitamin. Our experience has indicated that mice are not suitable animals for the detection of vitamin A by the methods at present in use.

The two sets of results with rats shown in table 6 are explicable if the initial weights of the animals used are taken into account. The four rats,

nos. 40, 41, 48, 49, which weighed 61 grams or less at the beginning of the feeding period, showed severe xerophthalmias, declined and died in nine to eleven weeks upon relatively large dosage of almond oil. Upon the same or larger amounts of almond oil three other rats, nos. 38, 39, 45, which weighed 81, 88 and 105 grams at the beginning, were able to survive the fifteen weeks of the experiment and to add reasonably large increases to their body weight. This discrepancy illustrated the truth of the statement by Zilva and Miura (26), Drummond and others that rats having a body weight of more than 90 grams are useless for accurate detection of

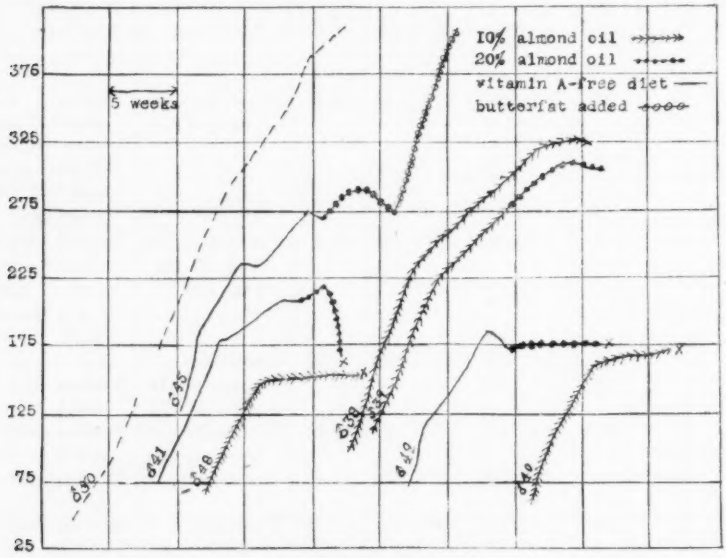

Chart 2. Growth curves of 7 rats fed almond oil as source of vitamin A

Basal diet and 0.3 gram dried brewery yeast daily were given in each case in addition to the almond oil. Rat 50, a litter mate of the other animals for which curves are given in this chart, was fed 0.3 gram yeast and basal diet containing 5 per cent butter fat instead of the almond oil.

vitamin A. Evidently the larger animals had stored enough vitamin A to carry them over the period of observation. When our work was discontinued all of these rats had begun to lose weight.

No greater concentrations of oil than 20 per cent were used in any of these feeding experiments, 5, 10, 15 and 20 per cent of the almond oil being substituted for corresponding amounts of crisco in the basal diet. In the curative tests the oil was given separately from the basal diet in 0.2 gram daily doses. No tests were made upon the fat-free residue of the

almond, but feeding experiments using this residue as source of vitamin A are in progress in this laboratory. The growth curves of the almond oil fed rats are shown in chart 2.

Reproduction of mice upon almond oil as source of vitamin A. Although mice were found unsatisfactory as test animals for the discovery of the vitamin A value of almond oil, an attempt was made to determine the effect of such a diet upon their fertility. Eight pairs of mice mentioned in table 5 were mated, when three to four months old, males and females on butterfat diet being mated in their own group, and the almond oil fed mice mated similarly. The diet was maintained unchanged. The four females on butterfat diet each gave birth to a litter of seven young, all of which were alive when born. Of the four females on almond oil diet two produced litters, of two and seven respectively, but only one nursed her young, and the other ate her litter at birth. This relative sterility of the group fed the vitamin-A-deficient diet is in accord with the experience of Reynolds and Macomber (27), Evans and Bishop (28), and is borne out by the results of numerous other investigators.

Autopsy findings upon rats which died on almond oil diet. Rat 40 fed 10 per cent almond oil diet from weaning died after nine weeks, showed normal conditions except for the characteristic xerophthalmic film over one eye. Rat 48 fed vitamin-A-free diet till decline began, then 20 per cent almond oil, dead after eleven weeks, showed on autopsy severe xerophthalmia with corneal ulcers of both eyes. Rat 49 treated similarly to rat 41, dead after ten weeks, showed on autopsy films over both eyes. Rat 48 treated similarly to rat 40, dead after eleven weeks showed a diffused purulent condition originating in an abscess in the ear and jaw. This condition is mentioned by Falta and Noeggerath (29) as an occasional development from the conjunctivitis and panophthalmia found in rats. Rat 45 fed vitamin-A-free diet till decline began, was given 20 per cent almond oil, made slight gains for four weeks, then declined anew till it was obviously moribund. Upon administration of 1 gram of butterfat daily growth was renewed, 87 grams being added in two weeks.

SUMMARY OF EXPERIMENTS UPON THE VITAMIN A CONTENT OF ALMOND OIL.

1. Almond oil, obtained either by cold pressing or ether extraction, fed at a 10 per cent level does not prevent eye disease, decline and death in rats fed upon vitamin-A-free basal diet. It does, however, postpone such symptoms five to nine weeks beyond the time at which they uniformly occur upon the latter diet.

2. Almond oil fed at a 20 per cent level does not restore, except for short periods, rats which are declining in weight from lack of vitamin A, nor does it cure eye disease which has developed from the same cause.

3. Mice fed a diet containing 20 per cent of almond oil as source of vitamin A do not exhibit signs of vitamin A deficiency other than con-

siderably lowered fertility. The mouse probably requires for normal growth much smaller amount of vitamin A than other varieties of animals heretofore tested.

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DAILY VARIATIONS IN CARDIO-VASCULAR CONDITIONS AND A PHYSICAL EFFICENCY RATING

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Purpose and plan. Use is made, in a physical fitness test proposed by Schneider (10), of certain cardio-vascular data which are assumed to be readily available if certain precautions are taken to eliminate well-known disturbing causes. The data employed are the pulse rates during recumbency and standing, the increase in the frequency of the heart beat when the standing and reclining postures are compared, the acceleration of the pulse rate by a standard exercise, the time required for the pulse rate to return to normal after the exercise, and the change in the systolic arterial blood pressure when a change is made from recumbency to standing. It is believed that a slow heart rate in recumbency and standing, with a small difference between the two; a slight acceleration of the heart rate in exercise, with a quick return to normal afterward; and a rise in the systolic arterial blood pressure on standing, are excellent health signs and that in the physically fit individual the heart and vasomotor system are, so to speak, tuned to a different pitch than in the unfit or unhealthy person. The use of such factors as the above necessitates an understanding of the causes that temporarily disturb the normal level of action of each. It has been our purpose to investigate these causes of disturbance and more especially to find the diurnal variations, that may be considered as rhythmic, and the extent of these when present.

Our observations have been made on 9 women and 10 men for a total of 25 series. Each series comprised hourly observations on each of the cardio-vascular factors over a period of 12 to 24 consecutive hours. In 17 the subjects ate the usual meals, while those under observation for 24 hours in addition ate a lunch during the night, between 2 and 3 a.m. In the remaining series of 8 cases the subjects fasted, going without food from supper time one day until evening of the next. In these fasting experiments the observations began at 8 or 9 a.m. and extended through the following 12 or 13 hours. The members of the fasting group remained unusually quiet, guarding against physical exertion. One of these subjects, however, found it necessary to do some physical work between 9 and 10 a.m.

The members of the normal group carried on the ordinary routine of their sedentary occupations in the laboratory and at the desk. At the end of the day's work they were taken by automobile to the place of the night observations. The habitual smokers were permitted to smoke as usual but were required to do their smoking just after being tested rather than just before an observation.

The procedure followed in each case was first to have the subject recline for 5 minutes; then to count the pulse rate, continuing the count until it was constant; and next to determine the arterial blood pressures with a Tycos sphygmomanometer by the auscultation method. Following this the subject took the standing posture and stood at ease for 2 minutes, after which the pulse rate and arterial blood pressures were again determined. Finally, the subject took a standard easy exercise which consisted in stepping upon a chair 18½ inches high 5 times in 15 seconds as timed by a stop watch. The pulse rate was counted for 15 seconds immediately the exercise ceased and then the time required for the rate to return to normal was determined.

The data have been analyzed and means determined separately for the fasting and normal groups. For the establishment of the diurnal curves of the normal group we used the 13 cases for whom the data were most complete and satisfactory. Since the several meals were not eaten by all of the subjects at exactly the same time of the day, we have grouped the data according to the time of the meals rather than exactly by the clock. Thus the dinner was eaten between 6 and 7 by some subjects and 7 and 8 p.m. by the majority. For calculating the means the data were tabulated by corresponding hours up to the time of the meal and after the meal. Hence, in the curves of figure 2, prepared from the group designated as normals, the indicated time of day is only approximate. The lunch time was about the same for all, so that part of the curves is more exactly placed. The method used in determining the curves makes the mid-period of 1 and sometimes 2 hours between the meals the most uncertain part of the curve, but this in no way falsifies the curves. In some of the hourly records individual cases had to be omitted because the data had not been secured. When it was necessary to drop out cases, instead of determining the mean by the usual method, we determined for each individual how much the factor had increased or decreased since the last hourly determination, then averaged these changes and added or subtracted this from the average of the last hour for which the data of all cases were complete.

The pulse frequency. The early students of this subject came to the conclusion that the pulse frequency is greater in the morning than the evening. Robinson (9) in 1732 took the opposite view but Knox (7) and Guy (2) presented evidence to refute his observations. Knox made a de-

tailed study of one subject, finding the average reclining posture pulse rate in the morning 62 and in the evening 56; the sitting average in the morning 78.3, in the evening 67; the standing average in the morning 90, in the evening 77. Guy found for the sitting posture an average difference of 10 betwen morning and evening. It was furthermore claimed that the heart was more excitable in the morning, food and exercise increasing the rate most in the early morning and forenoon, less in the afternoon and least of all in the evening. According to Knox, the change in posture from recumbency to standing increased the frequency in the morning by from 15 to 20 beats, at midday about 10 and in the evening 4 to 6 beats. Guy found that taking food might increase the rate for 2 hours in the morning and for a shorter time in the evening. Later writers, however, have accepted Robinson's view, that the pulse frequency is greatest in the evening. Hensen (3) in 1900 made observations on a healthy girl, who remained in bed, in whom an afternoon rise occurred independent of meals. Erlanger and Hooker (1) and Weysse and Lutz (11) reported an increase after the ingestion of meals and a general increase throughout the day.

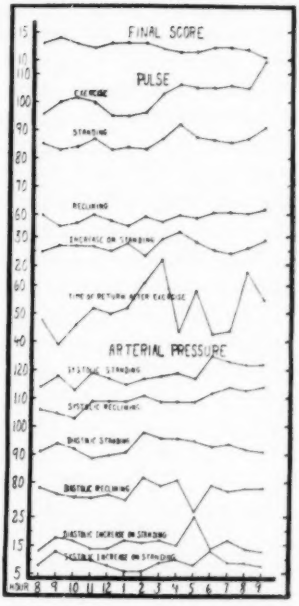

Fig. 1. Curves of means from hourly observations of fasting individuals.

If the morning and evening pulse rates vary as much as, and in the direction that the early writers thought, then any scheme which uses the postural and exercise pulse rates as evidence of the physical condition of men must make allowance for these differences that are not associated with fatigue. Our own observations, however, lend support to the more recent teaching that there is a general increase in rate throughout the day, and fail to indicate a decided diurnal change in the excitability of the heart.

In the 8 series of observations on persons who went without food from supper time of one day until evening the next, we saw no instance of a decrease in the pulse rate with the passing of the day. For the 4 cases in which all the circulatory data are complete generalized curves have been prepared and are given in figure 1. With this group the first observation was made at 8 o'clock in the morning and the last at 9 o'clock

in the evening. The curve for the reclining posture rate shows a heart frequency in recumbency practically unchanged with the passing of the day; although a slight increase is present during the last 5 hours. The same condition was true for the other group of 4 cases, 2 men and 2 women past middle age. In the standing posture there was a clearer tendency toward an increased rate in the afternoon; the group average for the young people rose from 85 at 8 a. m. to 87 at 8 p. m. and 91 at 9 o'clock; the older group average rose from 74 at 9 a. m. to 81 at 5 p. m. The rate after the standard exercise was only determined with the young group. Their average exercise rate (see fig. 1) rose from 99.5 at 8 a. m. to 105 at 8 p. m. and 115 at 9 p. m. Therefore, it is evident that the change that did occur was in the direction of an increased rate as the day passed, and that this may be looked upon as one of the body rhythms. No change in the excitability of the heart was indicated in the acceleration due to taking the standing posture, the first five observations in the morning gave an average difference between standing and reclining of 26.2, and the average of the last five observations of the evening was 26.8. The heart accelerated slightly more in exercise during the latter part of the day than during the morning. The average acceleration following the standard exercise for the first five morning observations was 14.5 beats and for the last five of the afternoon 19.7 beats.

The influence of the ingestion of food was observed in the 13 subjects who were examined hourly for 24 consecutive hours. From the generalized curves obtained from these data, which appear in figure 2, it will be observed that the noon lunch increased the recumbency pulse rate an average of 11.3 beats, the standing rate 8.1 beats and the exercise rate 7.1 beats; that the evening dinner increased the recumbency rate 11.7, the standing rate 10.2, and the exercise rate 13.1 beats; the night lunch increased the recumbency rate 6.9, the standing 5.7 and the exercise 6.4 beats. The data for breakfast were not very satisfactory in that more exercise was indulged in during this interval than during the remainder of the day; but even with that added disturbing factor, the increase in the frequency of the heart beat was not in excess of that observed after other meals. The after breakfast increase averaged for recumbency 9.3, standing 9.7 and after exercise 9.3 beats. Our data fail to prove that the heart is more irritable to the ingestion of food during one part of the day than another. There clearly is no evidence to support the belief that the morning is the period of greatest excitability. This is shown by a comparison of the standing-reclining and the standing-exercise differences before and after meals. Thus the average difference between the standing and reclining rates was before lunch 22.8, after lunch 19.6 beats; before dinner 17, after dinner 15.5; before the night lunch 21 and after the night lunch 19.8 beats. The standing exercise

differences were before lunch 11.6, after lunch 10.4; before dinner 11.1, after dinner 14.0; before the night lunch 8.5, and after the night lunch 7.2 beats.

The response of the pulse rate to the ingestion of food may be prompt or delayed as much as an hour. The effect lasts 2 or 3 hours, with occasionally a slight after-effect for still another hour.

The diurnal variations in the difference between reclining and standing pulse rates, for the group of 13 cases studied for 24 hours, are indicated in a curve in figure 2. There is a range of 7 beats in the difference throughout 24 hours, with the mid or zero condition in the morning, a maximum at noon and a minimum in the early evening. The difference between the standing and exercise pulse rate showed no constant diurnal variations. The average difference for periods of 5 hours in the morning was 11.1, afternoon 12.2 and night 12.2 beats,—from which no reason for the belief that there is a variation in irritability is indicated. For the fasting group the difference increased somewhat with the passing of the day because in standing there was a general rise in the frequency while no change occurred in recumbency.

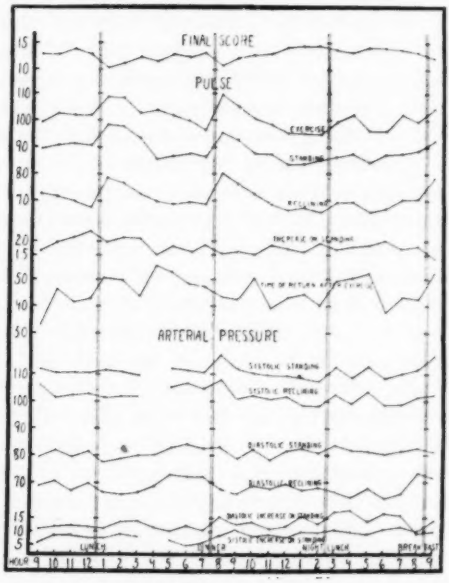

Fig. 2. Curves of means from hourly observations on individuals living normally.

One feature of the 24 hour pulse rate curves that was quite uniformly present was a gradual moderate retardation in the rate from about 8 p.m., to about 3 a.m., and then a slow acceleration which averaged 7 beats for the reclining, 5 for standing and 6 for exercise. The night slowing of the pulse rate was present even in those subjects who did not sleep. Of course our observations were not made during sleep. In view of this the fact, recorded by Klewitz (6), that during day sleep the rate is substantially higher than during night sleep, seems to find its explanation in the occurrence of a normal rhythmic diurnal retardation of the frequency of the heart beat.

The time required for the return of the pulse rate to normal after the standard exercise showed certain slight variations but not a clearly defined rhythmic diurnal variation. However, the curves for the fasting and normal groups given in figures 1 and 2 have in common an increase in the time of return from early morning until 3 or 4 o'clock in the afternoon and then a decrease until in the evening. Yet a study of individual cases, both during fasting and normal living, reveals many erratic fluctuations which could not be associated with any known cause. The range of the time of return in individual cases was ordinarily confined to narrow limits, in 8 of the 13 normal cases the range for the 24 hours was from 20 to 70 seconds. Two subjects ranged between 40 and 55 seconds. Other subjects, while on the whole reacting quite uniformly, occasionally without any observable cause required about 120 seconds to make the return. On the whole these are the least satisfactory of all our diurnal curves.

Arterial pressures. A review of the literature pertaining to the diurnal variations in the arterial blood pressure has been given by Weysse and Lutz (11). Most of the studies have considered only the systolic arterial pressure and were made on subjects who ate the usual meals and were busy with a work routine. Weysse and Lutz, from a study of 10 healthy male college students, conclude that the systolic pressure rises an average of 8 mm. immediately after the ingestion of food, then gradually falls until the beginning of the next meal; and that there is a general rise during the day. They found the diastolic pressure to be very uniform throughout the day and little affected by the ingestion and digestion of meals. A general tendency to a slight lowering of the diastolic pressure throughout the day was reported. A majority of the writers on this subject have observed the rise after meals and the general rise during the day. Erlanger and Hooker (1) believe it possible to explain the diurnal variations almost entirely by the effect of the ingestion of meals. Hensen (3), however, believes that there is a periodic rise in the systolic pressure independent of meals. Hill (4) found that the systolic pressure is higher after a hard day's work and that this pressure falls with standing when the subject is fatigued as, for example, after an all-night dance. Jellinek (5) studied 20 soldiers before and after meals, of whom 14 showed a rise in pressure, 2 a fall and 4 no change. Oliver (8) found a rise of 15 to 20 mm. after meals that attained its maximum in 1 hour and did not wholly disappear for from $2\frac{1}{2}$ to 4 hours.

Our data permit a comparison of the diurnal curves under two conditions, days without food and days in which food was taken as usual and in which the ordinary routine of laboratory and clerical work was followed. The arterial pressures were first separately compared for women and men. There were hourly observations made for 24 hours for 10 times on 7 women and 7 times on 7 men. The curves of means established from these data

were quite similar but on different levels for the two sexes. The average postural systolic pressure, during the 24 hours, fluctuated for recumbency in women from 95 to 107 mm. and in men from 109 to 120 mm.; and for the standing position in the women from 104 to 113.7, mm. and in the men from 116.5 to 123.5 mm. All of the pronounced fluctations of the pressure seemed to be due to the ingestion and digestion of food, which usually caused a sharp rise in the pressure. Individual differences were present and for the most part no two meals caused the same amount of rise. No clear cut sex difference other than that of the level of pressure was observed. The average diastolic pressure fluctuated, during the 24 hours, in recumbency for the women from 64 to 73 mm. and for the men from 65 to 73 mm.; and in the standing position for the women from 77 to 86 mm. and for men from 73 to 83 mm. Our results, contrary to the observations of Weysse and Lutz, appear to indicate that ingestion of food causes some change in the diastolic pressure. Our generalized curves of figure 2, prepared from the 10 cases of women and 3 of men, show that a rise in the systolic pressure almost invariably occurs after meals but that a change in one direction is not so certain to occur in the diastolic pressure. The most frequently observed response of the diastolic pressure was a food ingestion fall during recumbency and a rise during standing. The meal effect was not in evidence for so long a time upon the arterial pressure as upon the pulse frequency.

A general daytime rise in the level of the arterial pressures was not present in the group of cases that ate the usual meals. However, in the group that went without food the systolic pressure showed a gradual rise with the passing of the day. The data for these averages are plotted in figure 1. The reclining posture pressure rose gradually from 106 mm. at 8 a.m. to 114 mm. at 7 p.m., and the standing pressure from 114 mm. at 8 p.m., to 125 mm. at 6 p.m. The diastolic changes were not as uniform as those of the systolic pressure. In both postures the afternoon diastolic pressure was higher than in the morning, but the last determinations made at 7, 8 and 9 o'clock in the evening were again back to the early morning level. In neither of our series was there any such tendency toward a lowering of the diastolic pressure with the passing of the day, as Weysse and Lutz observed. For the normal group the diastolic pressure during the night was slightly lower than during the day time in recumbency.

The pulse pressure reflected the changes in the systolic pressure. Consequently, the diurnal curves for the pulse pressure show a gradual rise throughout the day.

Since it is believed that the systolic pressure falls with standing when a subject is fatigued, due to the failure of the splanchnic vasomotor compensation, the diurnal changes in the difference between the systolic pressure in the erect posture and recumbency were studied. In the fasting

group no general diurnal change was present during the 12 hours of observation. The members of the group differed greatly, 4 always had a rise in pressure on standing, one constantly showed a fall of varying degree, while the others fluctuated up and down in unaccountable fashion. With the normal group the curve (see fig. 2) for the difference between reclining and standing showed a rise after the evening meal to a level that was higher than during the day time and this was then maintained quite uniformly throughout the night. A tendency to the lowering of the recumbency systolic pressure was present during the night and this in part explains the greater rise on standing. In individual cases there were times when the usual systolic pressure rise did not occur with standing and the pressure even fell slightly; but we were unable to associate the condition with any circumstance, such as the state of the digestive canal, exercise or external temperature.

The efficiency index. The curves of averages of the final score of the six factors as rated by the Schneider index (10) have been plotted for the 2 groups of cases. The perfect score for this scheme of rating is 18. For the fasting group a gradual moderate fall of 3 points occurred throughout the 12 hours of observation. With the normal group a sharp fall occurred after each meal which lasted from $1\frac{1}{2}$ to $2\frac{1}{2}$ hours. There was also a tendency for the score to be slightly lower during the afternoon than during the morning hours. There was, furthermore, a clearly defined rise in the score during the night which, except for the two determinations that followed the night lunch, was continuously above the day portion of the curve. In those cases in which the influence of meals was eliminated the final score did not vary more than 3 or 4 points throughout the day; while, when meals were taken, the variations in several instances were as high as 10 points. The ingestion of food invariably lowered the score, chiefly by accelerating the pulse frequency in both the reclining and standing postures, but mostly in standing and after exercise. Frequently the systolic arterial pressure fell after meals and when it did not the rise that occurred was less than ordinary.

There are a number of things that may temporarily lower the score in addition to the ingestion of food. The after-effects of physical exertion have lowered it by as much as 10 points and in some instances these lasted for 2 or 3 hours. Smoking, especially in those not accustomed to tobacco, causes a lowering for 30 to 50 minutes which is largely due to a fall in the systolic pressure during standing. However, at the same time there is ordinarily a quickening of the pulse, especially in standing.

The night rise in the efficiency curve (see final score curve in fig. 2) is accounted for by the postural and exercise decrease in pulse rate and the greater compensatory systolic pressure rise on standing.

SUMMARY

Diurnal curves have been prepared from hourly determinations, during fasting and normal living, of the pulse frequency in recumbency, standing and after a standard exercise; the increase in the heart rate when the standing and reclining postures are compared; the amount of acceleration by exercise; the time required for the pulse rate to return to normal after exercise; the systolic and diastolic arterial pressures and the change in the systolic arterial blood pressure when the change is made from recumbency to standing.

During fasting the recumbency heart rate remained quite constant throughout the daytime, while in standing and exercise a slight general rise in rate occurred with the passing of the day. The ingestion of food invariably accelerated the heart frequency for 2 or 3 hours. On days when the usual meals were taken the tendency for an afternoon and evening increase in the rate was obscured. During the night the pulse rate slowed until 3 a.m. and then gradually returned to its normal tempo.

There was no clear cut evidence of a rhythmic diurnal change in the difference between the standing and reclining pulse rates. The time required by the pulse rate to return to normal after the standard exercise increased slightly until the middle of the afternoon and then decreased until evening. On the whole, however, unaccounted for fluctuations tended to obscure the diurnal rhythm in individual cases. The heart was not found to be decidedly more excitable one part of the day than another.

During fasting the systolic arterial blood pressure showed a general rise with the passing of the day but not when the usual meals were eaten. The diastolic pressure did not show a daytime rhythmic change but during the night it decreased slightly in recumbency. The systolic pressure rose more upon standing during the night than during the daytime. The ingestion of food regularly caused a rise in the systolic pressure and usually in the diastolic pressure.

The diurnal curve of the Schneider index was slightly lower for the afternoon than for the morning hours. There was also a well-defined rise in this index during the night.

For valuable aid in the determination of fasting conditions we are indebted to Mr. G. C. Ring. We also desire to thank those who served as subjects for these observations.

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SIMULTANEOUS CARDIOGRAPHIC AND ELECTROCARDIOGRAPHIC RECORDS IN MAN

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With the possible clinical application of measurements of pulse wave velocity in mind, it is very important to determine rising tension time in man under varying conditions, since it is often very difficult to interpret either heart sound or apex beat records in cases with abnormal conditions of the heart. While it is perfectly possible to measure pulse wave velocity in such cases in the peripheral vessels (e.g., by comparison of right carotid with brachial or radial), any such measurement leaves the elasticity of the aortic arch undetermined and there can be little doubt that not only is it the most elastic part of the vascular system, but also one very liable to pathological changes involving its elasticity. In such patients the R wave in the electrocardiograph and the upstroke of the brachial pulse are generally very definite points and it would be possible to make approximate measurements of the pulse wave velocity if the rising tension time were at all constant.

The experiments here detailed were designed to determine how much rising tension time might vary in the same subject under different conditions and between normal and abnormal conditions, and for this purpose simultaneous records of electrocardiograph, apex beat, heart sounds and brachials were taken photographically. While these experiments were designed for the measurement of rising tension time and the results mostly seem to confirm Weitz's interpretation of apex beat records, one of the most interesting and definite results has been a very noticeable variation of the time relations between the end of T and the second sound under different conditions.

General method. The mechanical records were taken with Frank capsules (Wigger's modification) and the heart sounds also by the method described by Wiggers and Dean (1). The tracings were measured by a Keith Lucas comparator and corrections made for parallax (when present) and for the differences in conduction rate of electrical and mechanical records. Measurements of the length of the arm were made as described by Bazett and Dreyer (2).

An indication for the end of the rising tension time and the beginning of the expulsion period was sought by the comparison of sounds at the

base with an apex beat record and by examination of the sounds at the apex.

The initial sound vibrations described by Wiggers and Dean were in many cases very small in amplitude at the base and even unrecordable during the early stages of ventricular systole. When starting some while after the R wave it seemed reasonable to suppose that the sounds commenced with the expulsion period and corresponded to the second component of aortic sounds described by Wiggers and Dean in the exposed heart. Where sounds were recorded at the base starting at about the peak of R or earlier there was usually a considerable change in their amplitude or frequency at a point about the same distance after the R wave as the origin of the first sound in those cases where the initial vibrations were not seen. In these records the origin or change in the first sound at the base usually showed a very close correspondence with a change in the apex beat although this change was not always of the same character in a series of records.

In some records sounds were recorded at the apex and the time at which there was a definite change in the amplitude or frequency of the vibrations was noted and this time had a rough correspondence with the origin of the first sound at the base when this was delayed. It seems probable that any point on the record which shows a considerable change in the character of the sounds at the base and to a less degree at the apex and which is also associated with a considerable change in the apex beat is likely to depend on a change from the isometric to the expulsion period.

An exact correspondence between the different records was not, as a rule, found but this is not surprising in view of possible experimental errors and also of possible variations between the right and left side of the heart, since it is unlikely that the rising tension time is always exactly the same in the two chambers. It will be seen that in some records it is very difficult to decide between two or three points, but the variation in the rising tension time produced is rarely more than 0.02 second whichever point is taken and in many cases there seems to be only one possible point which could indicate the change to the expulsion period. The records in abnormal cases are often very difficult to interpret, but if the rising tension time is to be determined at all it is probably less difficult by apex beat records than by sound records, since of the two the apex is the least liable to be so changed as to be undecipherable.

The onset of the second sound is very definite in almost all records and it became very noticeable that the relationship of this to the end of the T wave may vary considerably even in the same subject. The relationship of this to the second sound was very similar whether the comparison was made between the end of T and the apex beat record or with the true sound record, although the latter was much the clearer. The figures

given in this paper are averages taken from at least three cycles in all subjects while in certain cases many more than this have been read and averaged.

Results obtained in normal subjects. It soon became clear that over and above variations due to experimental error others seemed to exist from cycle to cycle in the same subject. Records were therefore taken from one subject with sinus arrhythmia to determine whether respiratory variations of the circulation determine some of these variations. In this subject readings were made of 37 cycles varying in duration between 0.637 and 1.200, records being taken of sounds at the apex, sounds at the base, apex beat, brachial and electrocardiograph. A record was also taken of pressure changes at the base, but was not found readable. Figure 1A shows a record of the electrocardiograph, brachial, and sounds at the apex. In the sound record an increase in amplitude occurred on the average 0.036 second after the peak of R and this time did not vary much between the long and short cycles. Figure 1B shows sounds at the base with an apex record from the same subject taken on the same occasion, and with this arrangement of the tambours the commencement of the expulsion period was measured as occurring, as judged by the sound record, between 0.034 and 0.054 second after the peak of *R* with a general average of about 0.04. The apex beat showed a change (marked *E* on the record) which seemed to correspond closely with the above change in the first sound and the character of the apex beat was fairly constant in all records. Averaging the results obtained in all heart cycles of the duration of 0.85 or above and comparing with those which were shorter than 0.85, averages of 16 to 21 cycles were obtained for the two groups and the rising tension time was similar in the two sets, but was slightly longer in the shorter cycles. There was also a lengthening of about 0.01 in the interval between the peak of R and the brachial in the short cycles as compared with the long ones. These differences were rather more marked if the extremes in the cycles were compared with one another. They are probably real, although in the comparison of individual cycles the possibility of experimental error makes the results less reliable. Since the change for the time from the R to the brachial was greater than that in the rising tension time estimated from the apex beat, it is clear that the time of transmission of the pulse wave from the heart to the brachial is also slightly longer in the shorter cycles. There was also noticed a slight change in relation of the end of T to the second sound, the second sound occurring rather earlier after the end of T in the short cycles. The differences seen in this subject with sinus arrhythmia are shown in table 1.

There are therefore variations with changes in cycle length occurring in sinus arrhythmia.

The differentiation of the actual point measured may perhaps be considered more easily in connection with records from other subjects. Figures 2 and 3 are enlarged reproductions of a small sections of records from two normal subjects. (For figs. 1, 2 and 3 the correction for the transmission time of the mechanical records is 0.005 second. In all the later figures the correction is 0.008 second.) In these figures the apex beat records have been outlined with white ink so as to distinguish them from the sound records, where the two records cross. It is clear in figure 2 that some slow initial vibrations of small amplitude start in the sound record at about the time of the peak of the R wave, and a little later during the upstroke of a slow wave a sharper vibration of larger amplitude is suddenly superimposed. This change occurred about 0.026 second after the peak of the R wave. The record was taken immediately after exercise, and the pulse rate at this time was 86. It seems unlikely

TABLE 1

	CYCLE	R TO 1 ON 1ST SOUND	R TO E ON APEX	R TO BRACHIAL	T TO 2ND SOUND
Average of 21 short cycles	0.761	0.039	0.045	0.143	0.027
Average of 16 long cycles	1.009	0.038	0.041	0.135	0.032
Figures of 4 consecutive cycles	0.692		0.046	0.182	0.034
	0.744	0.048		0.156	0.028
	0.840	0.044	0.042	0.158	0.027
	1.197	0.036	0.033	0.130	0.024

Subject, age 29, distance heart to brachial 43 cm., B. P. 105 to 102/68 to 72.

that this change in the sounds at the base could indicate the beginning of the mechanical change since it is so late after the peak of R, and it occurs about 0.08 second after the start of the Q wave. The most reasonable explanation seems to be therefore the beginning of the expulsion period. It will be seen there is a marked change in apex beat at the same time. The apex beat shows a small rise during the upstroke of R probably corresponding with auricular changes (compare Wiggers and Dean on initial vibrations for heart sounds) and then two small waves starting near the peak of the R and lasting until this change which we have marked *E* in the record. The small vibrations probably would correspond with the heart sounds during rising tension time. At the point *E* there is a great increase in pressure which is most reasonably explained by the coming forward of the apex through the straightening of the arch of the aorta as it is distended with blood, and this change occurs at the same time as the alteration in the sound record.

Figure 3 is a simpler record from another subject. In this record also there is a definite change in the first sound marked *1* on the record and

this occurred 0.041 after the peak of R. This record also was taken after the exercise and the subject's pulse rate at the time was 83. The apex beat in this record shows a slow rise of pressure due to auricular contraction and then a smaller wave starting at the peak of R and lasting over with a smooth outline until a point corresponding with point *1* on the sound record, when the apex beat record rises with some vibrations on the upstroke. Weitz (3) has described the common occurrence of two notches due to sound vibrations occurring at the point at which the apex beat changes, according to his interpretation, from the rising tension time to the expulsion period and he thinks that of these vibrations the first belongs to the rising tension time and the second to the expulsion period. By comparison in our records of the apex beat with the first sound at the base this interpretation is usually substantiated. Where only one of these secondary waves is seen it usually seems to belong to the expulsion period.

In the record reproduced in figure 4 the middle tambour shows heart sounds with some pressure changes. In order to simplify the taking of tracings a fairly long rubber tube was used, so that to obtain records the side tube had to be partially closed and a mixed pressure and sound record was obtained at the base; it is clear, however, that sound changes begin at the point marked *1* on the record and it is difficult to believe that this is anything else but the beginning of the expulsion period, since these vibrations occurred 0.042 second after the R wave. The only other possible point for the start of the expulsion period appeared to be the considerable downstroke in the curve due to pressure changes starting somewhat later and this would make the rising tension period almost unbelievably long. It will be seen on the record that the point marked *E* on the apex beat curve corresponds very closely with point 1 of the sounds at the base (0.039 after the peak of R). The close correspondence of these two points indicates a very definite change in the heart at this time and probably that from the isometric contraction to the expulsion period. A comparison of the four cycles shows how the apex beat may vary and it will be noticed that the point *E* is the second large upstroke of the apex beat curve or a small wave just preceding this, as for instance in the last cycle reproduced. During the supposed rising tension period the apex beat in this subject usually shows one notch, probably a heart sound vibration, and during the expulsion period one or two may be recognized. Such an interpretation of this curve agrees with Weitz's conclusions, but other cases occur in which the first sound does not seem to correspond with these points on the apex beat.

Figures 5A and B show curves obtained in a subject before and after exercise. In figure 5A taken before exercise there is a sharp upstroke on the sound curve which occurs about 0.023 after the peak of R and 0.063

after the beginning of R. This point seems rather early for the start of the expulsion period but also rather late for the commencement of the mechanical contraction. The apex beat shows a slight rise and steeper fall corresponding closely with the downstroke of the R wave and shows resemblances both to figures 2 and 3. By comparison with these figures the point *E* might be placed at either of the points marked *1* and *2*. Point one occurs 0.014, point two 0.040 seconds after the top of R, and it is the peak in between these two points which corresponds most closely with the onset of the first sound, this peak occurring 0.028 second after R as compared to 0.023 for the first sound. It does not seem possible that the rising tension time could end before point *1* and it is difficult to find any probable point later than point *2*. Point *2* is most probable by comparison with the rising tension time determined in this way from other normal apex beat curves, but it is impossible to decide this definitely. A conceivable explanation is that the sounds may be determined by the rising tension time of the right side of the heart and point *2* by the rising tension time of the left side of the heart under circumstances in which these two times are not identical, but the curves illustrate well the complexities that may arise. Figure 5A shows quite clearly that the second sound occurred quite definitely after the end of T, according to our measurements 0.025 second later.

Figure 5B is a record obtained from the same subject immediately after exercise, without change of the tambours, with a change in pulse rate from 64 to 114. The sounds shown on the record are as before with a sharp onset shortly after the R. The start of these sounds is now 0.017 after the peak of R. The apex is similar to that before exercise but the vibrations are somewhat differently placed. It would seem possible to time the end of the rising tension period at any one of the points marked *1*, *2* and *3* with *2* or *3* appearing the more probable. Point *1* is 0.013 after the peak of R, point *2* is 0.033, point *3* is 0.062. The commencement of the first sound corresponds most closely with the peak between point *1* and *2*, and there is a considerable change in the sounds (the record also showing a certain amount of pressure change) corresponding more or less with point *3*. In this particular record it does not seem possible to state what is the rising tension time. According to Weitz the end of rising tension time should presumably be indicated by point *2*. The pulse wave velocity to the brachial at the elbow after exercise if calculated from point *1* would be 4 meters per second, if calculated from point *2*, 4.9, if calculated from point *3*, 6.5 meters per second. The pulse wave velocity of the brachial while resting calculated from point *2* in figure 5A is 3.5 and it would be slower than this (2.9) if calculated from point *1*. This figure is reproduced to show the occasional difficulties in such records, but luckily such inconsistencies are rare, and even

so point *2* on each record would agree most easily with our general averages and would correspond with the point chosen by Weitz.

On the other hand, figure 5B showed quite clearly that after exercise the T wave outlasts the second sound considerably (even more when the correction for the transmission of the mechanical record of 0.008 is added) the actual time difference being by our measurements −0.024 second as compared to the +0.025 second found while resting. This difference is seen in spite of the fact that the T wave after exercise is less abrupt, so that in reading the record the tendency would be to minimize rather than exaggerate the change.

Effects of exercise. Attention has been drawn to the slight variations in the time relations that may occur with sinus arrhythmia, the greater changes induced in normal subjects by change of pulse rate as the result of exercise must now be considered in more detail. Several of the records already reproduced have been taken after exercise and in most cases (as in figs. 2 and 3) there seemed definite indications of the change from rising tension to expulsion period. The sounds were more difficult to interpret since as they became much louder the initial vibrations were nearly always recordable at the base, and it is not always easy to distinguish the change in the character of the sound waves. On the other hand the character of the apex beat is usually little altered. The rising tension time as measured in this way is shortened after exercise, the duration of R to brachial is also diminished, while the calculated pulse wave velocity to the brachial is the same or slightly faster.

There is a very definite change in the relation of the T wave to the second sound, since after exercise the T wave usually outlasts the second sound. In some experiments on dogs to be reported in another paper, Doctor Bazett and I have found that section of the vagus will produce just such a change, the T wave after the section outlasting the second sound, so that mere removal of vagal inhibition can produce this result. It has been shown by Wiggers that injections of adrenalin also produce such an effect, so that it seems probable that sympathetic stimulation can also be a factor. The change in the relationship of T to second sound seen from cycle to cycle in sinus arrhythmia would be then explicable as a result of simple variations in vagal tone. The animal experiments referred to above suggest that even mere excitement may induce this change. The whole question will be discussed again in connection with these experiments.

Table 2 shows the average relationship of this second sound to T and also the figures we have obtained for rising tension time both before and after exercise and it will be noticed that the changes with exercise are quite definite, although it is probable that if too long a time elapses after exercise there may be a swing-back so that the effect may be opposite to that seen earlier.

A few examples of these may be quoted with regard to the relationship of T to second sound. One subject had a second sound occurring 0.029 after the end of T while resting, and after exercise this was reduced to 0.023, while after sitting still for a short while the interval lengthened to be even longer than before exercise, namely, 0.037. In another subject the T outlasted the second sound and the difference was −0.004 even while resting, but directly after exercise this changed to −0.027 and after sitting a little while the difference was reduced to −0.010. A gradual change is seen, therefore, although in this subject in all three records the T wave outlasted the second sound. The average, maximum and minimum figures obtained are given in table 2.

TABLE 2

	R TO CHANGE IN 1ST SOUND	R TO APEX (E)	R TO Br	P. W. V. CALCULATED FROM SOUNDS IN METERS PER SECOND	P. W. V. CALCULATED FROM APEX	T TO 2ND
Resting:						
Maximum	0.058	0.051	0.188	5.7	5.6	+0.045
Minimum	0.017	0.023	0.081	2.3	2.4	−0.027
Average	0.036	0.035	0.143	3.8	3.9	+0.013
After exercise:						
Maximum	0.047	0.057	0.158	6.1	6.4	+0.037
Minimum	(0.009*)	0.013	0.105	2.4	2.3	−0.045
Average	0.025	0.033	0.127	4.1	5.0	+0.002

Of 22 resting times for T to 2nd 17 were plus and 5 minus.
Of 17 after exercise times for T to 2nd 7 were plus and 10 minus.
*A doubtful figure.

The variations in the rising tension time that are seen in the figures for rest are no doubt partly dependent on experimental error, since in certain subjects the point chosen for rising tension time on the sound record was not the same as that taken from the apex beat. The two figures, however, rarely differ by more than 0.015 second and in many cases the readings were identical. The commencement of the expulsion period at rest usually occurred between 0.030 second and 0.045 second after the peak of R, 57 per cent of the sound record figures and 68 per cent of the apex beat figures lying within this range.

The variations seen in the figures obtained after exercise are larger but this is not surprising in view of the rapid changes known to occur in the circulatory system after exercise. The discrepancy that is seen in this part of the table between the figures for rising tension time as determined from sound and apex beat records is partly due to measurements

of these points on different pulse cycles, since the violence of the changes would sometimes bring the light beyond the range of the paper, so that it was often impossible to read all the records in any one cycle. It is also partly due to the difficulty of reading the sound records after exercise, and the apex beat figures are probably the more reliable. But again in many cases there was an exact agreement between the two methods.

The relationship of T to the second sound is well brought out by the table and the variations as the result of exercise are clearly evident. Weitz in one of his papers (4) gives times from R to T and from R to the "Aortenklappenschluss" by comparison of which the duration of T to "Aortenklappenschluss" may be calculated. This difference gives an average of 0.006 from the end of T with variations even at rest from +0.072 to −0.078. His general average is close to my figure and in one case he took records both before and after exercise and where this interval was 0.033 before exercise, after exercise it was reduced to zero. His figures therefore agree with mine, although for some reason or other his variations are even larger than mine. For rising tension time also the figures I have obtained are in good agreement with those of Weitz, the main difference being that I have not attempted to state the actual durations of the rising tension time as he did, since it is often very difficult in the cardiograph to decide where the waves produced by the auricle end and those produced by the ventricular contraction commence. From the point of view of measurements of pulse wave velocity it is the commencement of the expulsion period which is important and the relationship of this to the R wave is more valuable than any measurement of rising tension time from the apex beat record itself. Where Weitz gives 0.06 duration for rising tension time he is taking the mechanical contractions as starting on the upstroke of the R wave, so that the time from the peak of the R wave should be less than this and would have to be about 0.04 or 0.05.

In the figures I obtained on some students previously and which were included in Bazett and Dreyer's paper, the pulse wave velocity was estimated from the electrocardiograph in some of the cases by allowing 0.06 second for the rising tension time measuring from the R wave and the figures obtained in this way gave a rather faster pulse wave velocity than those obtained from direct measurements of apex beat. The selection of this time of 0.06 second by these authors was made as expressing a figure that was likely to be nearer the maximum rather than the minimum possible figure and therefore one which would tend to make the pulse wave velocity to the brachial appear faster rather than slower than normal, so bringing the results as close as possible to previously reported figures. But the above discrepancy emphasized the need of more accurate values for this time. The average figures of the pulse

wave velocity to the brachial obtained in this series from sound and apex beat measurements suggest that the normal velocity is, if anything, even slower than the figure given by Bazett and Dreyer.

Records from abnormal cases. A few figures were obtained in patients with arteriosclerosis and also some with aortic regurgitation, although these cases were investigated more from the point of view of pulse wave velocity than for any particular relationship of mechanical to electric changes in the heart. The actual character of the curves varies considerably, varying even in the same subject on different occasions, but several of the types described by Weitz for abnormal hearts were seen. The points on the apex beat curves so obtained were compared with sound records, although with the presence of murmurs this was difficult and it was clear that in cases with heart lesions the sounds could rarely be used for determining the expulsion period. The apex beat curve in aortic regurgitation cases is very distinct in its character as compared with the normals. The auricular elements in the curve are often exaggerated and it is not uncommon to have sharp upstrokes resembling the changes produced in normal people by ventricular action starting even before the Q wave. This has also been observed in the experimental dogs already referred to with aortic lesions, which will be described in another paper. In spite of these abnormalities there is usually a sharp upstroke on the curve which occurs shortly after the peak of the R wave and seems to be entirely comparable to the upstroke on the normal curves taken to indicate the start of the expulsion period. The second sound could often be read on the tracings in spite of the murmurs and fairly reliable figures could be obtained for the relationship of the end of T to this sound, and these figures were checked by comparing with the end of ventricular systole as estimated by a rapid upstroke in the apex beat curve.

The figures obtained for arteriosclerosis cases give an average time for the commencement of the expulsion period of 0.049 after the peak of R and the second sound occurred 0.016 after the end of T. This gives a rising tension time of 0.01 of a second longer than the normal, while Weitz's figures also give an average of 0.074 as against a normal 0.06 for the total period of rising tension time.

The aortic cases (6 in number) give an average for the interval between the end of T and the second sound of -0.004. The average rising tension time lies between 0.030 and 0.035 according to the point taken in two cases which are somewhat anomalous. This gives a rather greater shortening of the rising tension time measurements than Weitz found in aortic insufficiency. The average of his figures is only 0.003 below his normal figures.

It will be noticed that there is quite a contrast in these cases between the normals and the aortic regurgitation subjects in a relationship of T

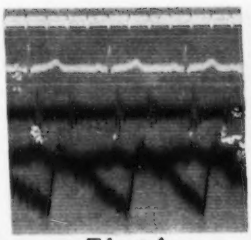

Fig. 1 A.

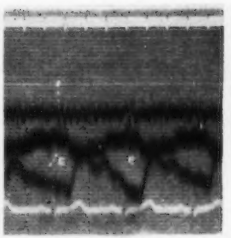

Fig. 1 B.

Fig. 2.

Fig. 3.

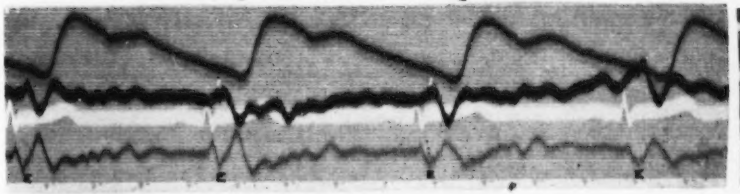

Fig. 4.

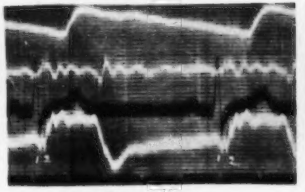

Fig. 5 A.

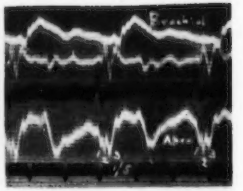

Fig. 5 B.

Fig. 1 A. Electrocardiograph, sounds at the apex, pressure record at the base and brachial from above downwards. Time marker ⅕ second. Pressure record at base not readable. Change in first sound marked *1* on the record. Transmission time of mechanical record 0.005 second.

B. Same subject. Sounds at base, pressure changes at apex, brachial and electrocardiograph from above downwards. Change in sounds at base marked *1* and point read on apex beat marked *E*.

Fig. 2. Sounds at base, pressure at apex in normal subject after exercise. Enlargement of part of the record and R wave. Brachial only partly shown. Apex beat outlined in white. Point read on apex beat mark *E*.

Fig. 3. Similar to figure 2 but another subject. Point read on sounds marked *1*. Point read on apex beat marked *E*.

Fig. 4. Brachial sounds at base with some pressure changes, electrocardiograph and apex beat from above downwards.

Fig. 5. Brachial, sounds, electrocardiograph and apex beat from above downwards. *a*, before exercise; *b*, after exercise.

to second sound although the arteriosclerotics give an average close to the normal. In the aortic cases there were only six subjects in which arteriosclerosis could be excluded, the lesions being apparently rheumatic, and it is these cases that are here considered. The actual figures in these cases vary between +0.020 and −0.029, the consistent negative figures being obtained in a girl of 23 with a recent valve lesion apparently with compensation developed or developing at this time. One case of heart block with a pulse rate of 30 was also examined. This was a patient aged 70 with marked arteriosclerosis. The rising tension time corresponded closely with other figures for arteriosclerosis, but the T wave always outlasted the second sound, the average figure being a −0.109 for the T to the second sound interval; in fact the second sound occurred near the peak of the T wave on its upstroke and the T was a sharp definite wave with a potential of about 0.3 mv. Such a striking discrepancy between the electrical and mechanical records needs explanation, but it was quite definite, since in most cycles the P waves did not interfere with determination of the end of the T wave.

CONCLUSIONS

1. In taking simultaneous records of heart sounds, apex beat and electrocardiograph, there is a close correspondence between changes in the first sound recorded at the base and an abrupt change in the pressure record recorded from the apex. This point occurs about 0.040 second after the peak of the R wave in normal cases and presumably indicates the start of the expulsion period. In many cases this point represents the commencement of sounds at the base and is quite definite.

2. The second sound occurs shortly after the end of the T wave in most normal cases during rest (as has been previously described), but if records are taken immediately after exercise the T wave usually considerably outlasts the second sound.

3. In sinus arrhythmia the end of the T wave precedes the second sound by a shorter time when the heart is beating faster than when it is slower.

4. In sinus arrhythmia the rising tension time is almost constant but, if anything, is longer rather than shorter with the quicker pulse.

5. From records of abnormal subjects the rising tension time is increased by about 0.01 second in cases of arteriosclerosis and diminished by almost the same amount in cases of aortic regurgitation of rheumatic origin. The aortic regurgitation cases also had a T wave which more commonly outlasted the second sound than in normal cases. There is no certainty that the Q-T or S-T interval will in any case correspond closely with the duration of mechanical systole and the discrepancy can be considerable.

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STUDIES IN PULSE WAVE VELOCITY

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Measurements of normal velocities from apex to femoral and dorsalis pedis. As there are in the literature few figures for pulse wave velocity from the heart to the femoral and dorsalis pedis these observations were made to establish normal values. Twelve normal young men students at the University were examined to determine this. With the patient recumbent, the measurement is taken from the second costal cartilage in the midline to a point opposite the fourth lumbar vertebra and then to the stem of the femoral and dorsalis pedis tambours. Post-mortem measures were made of vessels on four bodies and measurements as described above need on the average an addition of 13 cm. to allow for the length of the aortic arch when good agreement between the external measurement and the actual vessel length was obtained. A constant addition of 13 cm. was therefore made to the external measurements.

All examinations were made with patients in the recumbent position and records were taken optically, using Frank capsules. The blood pressure in the upper extremity was estimated by means of a Faught sphygmomanometer by the auscultatory method. The following results as tabulated below were obtained. Each figure in the columns of the table represents an average of four readings taken from the tracing of the individual case. Figures from the R wave are included so that any discrepancies in reading the apex beat may be controlled. For these determinations the rising tension time has been taken as lasting 0.040 after the peak of R, as described by one of us in a previous paper (2).

In the article of Bazett and Dreyer (1) on the "Measurements of Pulse Wave Velocity" the figures for the average pulse wave velocity of apex to brachial of 4.0 meters per second and brachial to radial of 8.8 meters per second are given. It is of note that these figures are comparable to ours for the central vessels and the peripheral vessels of the lower extremity. Comparing them it is seen here again that the rate over the larger vessels is slower than that of the smaller vessels.

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Top of R or apex beat to femoral or dorsalis pedis, Frank capsules

NUMBER	AGE	ARM	PULSE RATE	PULSE WAVE VELOCITY				
				A-F	R-F	A-DP	R-DP	F-DP
1	20	130/70	75	4.7	5.7	6.3	7.1	8.4
2	26	122/73	72	3.4	4.2	4.8	5.4	6.8
3	18	138/98	84	4.6	3.7	5.3	5.6	8.1
4	23	109/66	72	2.6	3.4	5.3	5.7	11.2
5	26	136/74	96	3.6	3.7	5.4	5.2	7.5
6	19	122/80	72	4.0	5.0	6.4	6.0	6.9
7*	24	130/80	84	4.3	4.6	5.9	5.7	6.6
8	22	120/78	72	5.3	5.0	7.5	6.3	7.9
9	22	122/77	84	3.2	4.0	4.6	4.7	5.4
10	26	124/70	96	5.1	4.2	6.6	4.9	6.3
11	24	138/88	60	4.1	6.5	5.1	6.2	6.2
12	24		96	5.3	5.4	6.6	5.6	5.7
13	25		71	4.5	5.6	6.3	7.1	8.75
Average.....				4.2	4.7	5.7	5.9	7.4

* Mitral regurgitation, compensated.

In the article above mentioned one or two normal figures were quoted for the pulse wave velocities in the lower extremities which correspond roughly with ours.

It is somewhat remarkable in view of this variation with the size of the vessel that the pulse wave velocity should appear to be almost identical in the aorta and the brachial artery, since the heart to the femoral involves mostly the aorta. The figures, if anything, indicate a rather more rapid velocity from the heart to the femoral at the groin than to the brachial at the elbow. The velocity in the peripheral vessels is very similar, surprisingly so considering the length of large vessels included in the distance of groin to dorsalis pedis.

The average length from the heart to the dorsalis pedis was 147 cm. and the average time of transmission of the pulse wave 0.26. It is clear that in these cases the pulse wave would not be able to travel as far as the dorsalis pedis and be reflected back to the heart during the expulsion period during ordinary heart cycles.

CONCLUSION

1. Additional evidence (gained by examination of the velocities occurring in the lower extremities) strengthens the belief that the pulse wave velocity is slower in central vessels and more rapid in the peripheral ones.

a. The average pulse wave velocity for central vessels from the heart to the groin is probably about 4.5.

b. The average pulse wave velocity from the groin to the foot in normal young men is 7.5 meters per second.

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